







# THE JOURNAL OF METABOLIC RESEARCH

EDITED BY

FREDERICK M. ALLEN

with the collaboration of

THOMAS ADDIS  
FREDERICK G. BANTING  
LEWELLYS F. BARKER  
W. R. BLOOR  
H. C. BRADLEY  
A. J. CARLSON  
E. G. CONKLIN  
G. H. A. CLOWES  
H. M. EVANS  
N. B. FOSTER  
JULIUS FRIEDENWALD  
CASIMIR FUNK  
J. T. HALSEY  
PHILIP B. HAWK  
YANDELL HENDERSON  
ALFRED HESS  
HAROLD L. HIGGINS  
D. R. HOOKER  
R. G. HOSKINS

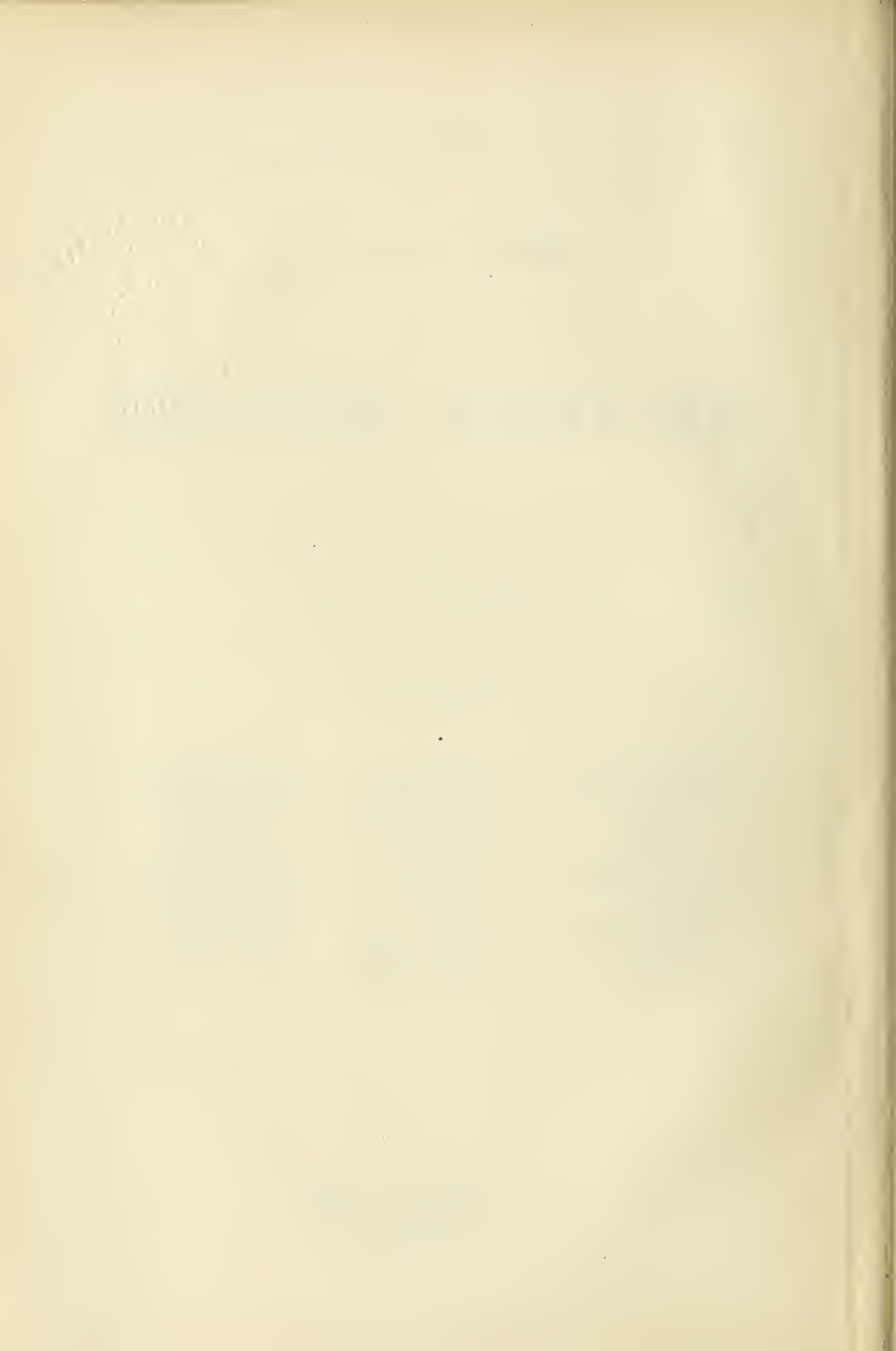
W. H. HOWELL  
HOLMES C. JACKSON  
E. C. KENDALL  
A. S. LOWENHART  
WILLIAM P. LUCAS  
A. B. MACALLUM  
J. J. R. MACLEOD  
WM. DE B. MACNIDER  
E. V. MCCOLLUM  
F. B. MALLORY  
DAVID MARINE  
W. McKIM MARRIOTT  
E. K. MARSHALL  
G. H. MEEKER  
EDWARD B. MEIGS  
JOSEPH L. MILLER  
JOHN R. MURLIN  
VICTOR C. MYERS  
L. H. NEWBURGH

J. E. PAULLIN  
FRANCIS W. PEABODY  
RALPH PEMBERTON  
H. S. PLUMMER  
L. G. ROWNTREE  
W. D. SANBURN  
P. A. SHAFFER  
H. C. SHIERMAN  
ALFRED STENGEL  
GEORGE N. STEWART  
JOHN P. STREET  
SOLOMON STROUSE  
FRANK P. UNDERHILL  
GEORGE WALLACE  
LEWIS H. WEED  
RAY L. WILBUR  
M. C. WINTERNITZ  
R. T. WOODYATT

195503  
17.4.25

Published Monthly by  
THE PHYSIATRIC INSTITUTE  
Morristown, New Jersey, U. S. A.

PRICE: \$10.00 PER YEAR



## INDEX—Volume 3

Acid-Base Equilibrium, studies concerning the influence of a disturbance in the—of the blood on renal function and pathology.	
Study 1. The effect of acid and alkaline solutions on renal function and pathology in normal dogs. MacNider, Wm. de B.	511
——— Study 2. The effect of acid and alkaline solutions on renal function and pathology in naturally nephropathic dogs. MacNider, Wm. de B.	539
——— Study 3. The ability of an alkaline solution to protect the kidney of normal and naturally nephropathic dogs against an acid solution. MacNider, Wm. de B.	569
Acidosis. 1. The production of diabetic acidosis and coma in dogs.	
Allen, Frederick M.	775
——— 2. Fat intoxication. Allen, Frederick M.	797
Adrenals, the relation of the—to diabetes. Allen, Frederick M.	589
Allen, Frederick M. Clinical observations with insulin. 3. The influence of fat and total calories on diabetes and the insulin requirement	61
——— Experimental studies in diabetes.	
Series 2. The internal pancreatic function in relation to body mass and metabolism. 11. The relation of the adrenals to diabetes	589
——— Experimental studies in diabetes.	
Series 2. The internal pancreatic function in relation to body mass and metabolism. 12. Diabetes and phlorizin glycosuria.	623
——— Experimental studies in diabetes.	
Series V. Acidosis. 1. The production of diabetic acidosis and coma in dogs.	775
——— Experimental studies in diabetes.	
Series V. Acidosis. 2. Fat intoxication.	797
Allen, Frederick M. and Sherrill, James W. Diet treatment of diabetes insipidus	479
Allen, Frederick M.; Weeks, David F.; Renner, Dan S., and Wishart, Mary B. Fasting and diets in treatment of epilepsy.	317
A Vitamine. To what extent is quantitative estimation of—possible? Holmes, Arthur D.	583

## INDEX - Volume 3—*Continued*

Amino-Acids in nutrition. VI. The nature of the supplementary value of protein-free milk to the total proteins of milk. Sure, Barnett .....	373
——— VII. Further studies on the cause of nutritive inadequacy of the proteins of the Georgia velvet bean ( <i>Stilzobium Deeringianum</i> ). Sure, Barnett.....	383
Barreto, A. L. B. The effect of X-ray exposure on metabolism.....	737
Basal Metabolic Rate, the after effects of prolonged fasting on—. Kunde, Margarete M.....	399
Best, C. H., and Scott, D. A. Possible sources of insulin.....	177
Bishop, Katherine Scott, and Evans, Herbert MacLean. See Evans. Blood pressure, non-protein nitrogen and—in relation to kidney and heart lesions. Floyd, Rolfe.....	759
Blatherwick, N. R., and others. Treatment of diabetes with insulin....	641
Cameron, Gordon. A comparison of Dodds' and Sladdens' methods for estimating urinary diastase.....	753
Carlson, A. C.; Eldridge, C. J.; Martin, H. P., and Foran, F. L. Studies on the physiological action of saccharin.....	451
Casein. The effect of purification of—on its food value. Funk, Casimir; Paton, Julia B., and Freedman, Louis.....	1
Chavarria, A. Pena.; Clark, J. H., and Evans, P. S. Jr. See Clark. Clark, J. H.; Evans, P. S. Jr., and Chavarria, A. Pena. Further studies on metabolism after exposure to X-rays.....	749
Cod Liver Oils. Studies of the vitamine potency of—. II. Vitamine potency of "spring" cod liver oil.....	393
——— IV. To what extent is quantitative estimation of vitamine A possible.....	583
Cohen, William, and others. Significance of phosphates in production of tetany .....	679
Cryst, J. H., and others. Treatment of diabetes with insulin.....	641
Davis, Marguerite. Effect of rations containing whole and skimmed milk on young growing puppies.....	711
——— Effect of various rations on young normal guinea pigs and on young guinea pigs inoculated with tuberculosis.....	725
Diabetes Insipidus, diet treatment of—. Allen, Frederick M., and Sherrill, James W.....	479

## INDEX - Volume 3—*Continued*

Diabetes. Experimental studies in—Series 2. The internal pancreatic function in relation to body mass and metabolism. 11. The relation of the adrenals to diabetes. Allen, Frederick M.	589
——— Experimental studies in—Series 2. The internal pancreatic function in relation to body mass and metabolism. 12. Diabetes and phlorizin glycosuria. Allen, Frederick M.	623
——— Experimental studies in—Series V. Acidosis. 1. The production of diabetic acidosis and coma in dogs. Allen, Frederick M.	775
——— Experimental studies in—Series V. Acidosis. 2. Fat intoxication. Allen, Frederick M.	797
——— The influence of carbohydrate and protein on—and the insulin requirement. Sherrill, James W.	13
——— The influence of fat and total calories on—and the insulin requirement. Allen, Frederick M.	61
——— Old and new treatment of diabetes from a statistical point of view. Heiberg, K. A.	677
——— The treatment of—with insulin. A report of the methods followed and the results obtained in the first one hundred cases. Sansum, W. D.; Blatherwick, N. R.; Smith, Florence H.; Long, M. Louisa; Maxwell, L. C.; Hill, Elsie; McCarty, Ray., and Cryst, J. H.	641
Eldridge, C. J., and others. Physiological action of saccharin.	451
Epilepsy. Observations of fasting and diets in treatment of—Weeks, David F.; Renner, Dan S.; Allen, Frederick M., and Wishart, Mary B.	317
Evans, Herbert MacLean, and Bishop, Katherine Scott. On the relations between fertility and nutrition. III. The normal reproductive performance of the rat.	201
——— The production of sterility with nutritional regimes adequate for growth and its cure with other foodstuffs.	233
Fasting. Observations on—and diets in the treatment of epilepsy. Weeks, David F.; Renner, Dan S.; Allen, Frederick M., and Wishart, Mary B.	317
——— The after effects of prolonged—on the basal metabolic rate. Kunde, Margarete M.	399
Fat. Influence of—and total calories on diabetes and insulin requirement. Allen, Frederick M.	61
Fat Intoxication. Allen, Frederick M.	797

# INDEX - Volume 3—Continued

Floyd, Rolfe. Non-protein nitrogen and blood pressure in relation to kidneys and heart lesions.....	759
Foran, F. L., and others. Physiological action of saccharin.....	451
Freedman, Louis; Funk, Casimir, and Paton, Julia B. See Funk.	
Funk, Casimir; Paton, Julia B., and Freedman, Louis. The effect of purification of casein on its food value.....	1
Germanium Dioxide, Effect of—upon the blood. Muller, John Hughes, and Iszard, Miriam Stewart.....	181
Gross, Erwin G., and others. Significance of phosphates in production of tetany.....	679
Heiberg, K. A. Old and new treatment of diabetes from a statistical point of view.....	677
Hill, Elsie, and others. Treatment of diabetes with insulin.....	641
Holmes, Arthur D. Studies of the vitamine potency of cod liver oils. II. Vitamine potency of "spring" cod liver oil.....	393
——— Studies of the vitamine potency of cod liver oils. IV. To what extent is quantitative estimation of vitamine A possible .....	583
Iszard, Miriam Stewart, and Muller, John Hughes. Effects of germanium dioxide upon the blood.....	181
Insulin. Clinical observations with—. 2. The influence of carbohydrate and protein on diabetes and—requirement. Sherrill, James W.....	13
——— 3. The influence of fat and total calories on diabetes and—requirement. Allen, Frederick M.....	61
——— Possible sources of—. Best, C. H., and Scott, D. A. ....	177
——— Treatment of diabetes with—. A report of the methods followed and the results obtained in the first one hundred cases. Sansum, W. D.; Blatherwick, N. R.; Smith, Florence H.; Long, M. Louisa; Maxwell, L. C.; Hill, Elsie; McCarty, Ray, and Cryst, J. H.....	641
Kunde, Margarete M. The after effects of prolonged fasting on the basal metabolic rate.....	399
Long, M. Louisa, and others. Treatment of diabetes with insulin.....	641
McCarty, Ray, and others. Treatment of diabetes with insulin.....	641



## INDEX - Volume 3—*Continued*

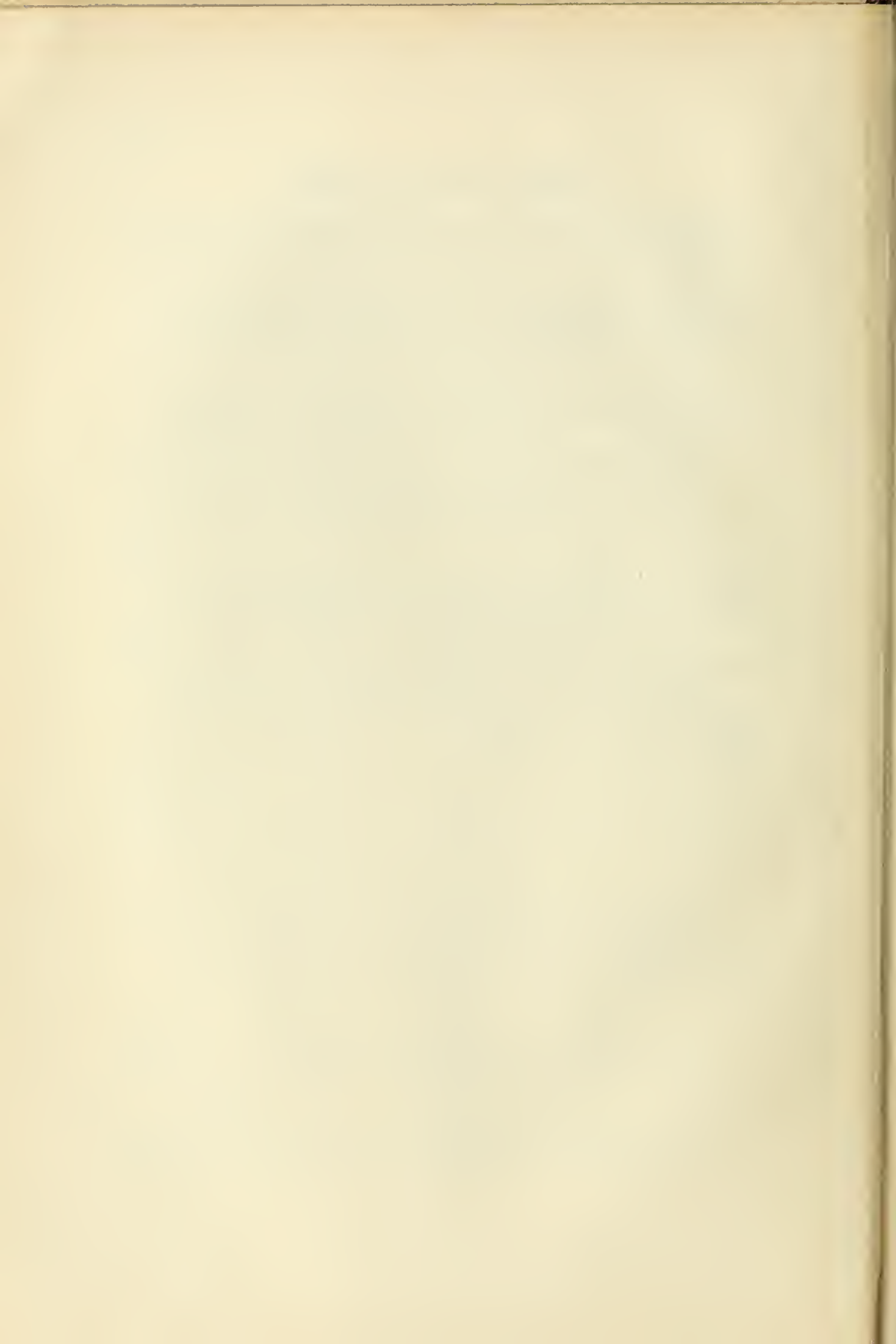
MacNider, Wm. de B. Studies concerning the influence of a disturbance in the acid-base equilibrium of the blood on renal function and pathology. Study 1. The effect of acid and alkaline solutions on renal function and pathology in normal dogs.....	511
——— Studies concerning the influence of a disturbance in the acid-base equilibrium of the blood on renal function and pathology. Study 2. The effect of acid and alkaline solutions on renal function and pathology in naturally nephropathic dogs .....	539
——— Studies concerning the influence of a disturbance in the acid-base equilibrium of the blood on renal function and pathology. Study 3. The ability of an alkaline solution to protect the kidney of normal and naturally nephropathic dogs against an acid solution.....	569
Maxwell, L. C., and others. Treatment of diabetes with insulin.....	641
Metabolism. The internal pancreatic function in relation to body mass and—. 11. The relation of the adrenals to diabetes. Allen, Frederick M.....	589
——— 12. Diabetes and phlorizin glycosuria. Allen, Frederick M. ....	623
——— Studies in inorganic—. Study III. The significance of phosphates in the production of tetany. Underhill, Frank B.; Gross, Erwin G., and Cohen, William.....	679
——— Further studies on—after exposure to X-rays. Clark, J. H.; Evans, P. S. Jr., and Chavarria, A. Pena .....	749
——— The effect of X-ray exposure on—. Baretto, A. L. B. ....	737
Milk. The distribution of sulphur in protein-free—. Sure, Barnett, and O'Kelly, R. E.....	365
——— The nature of the supplementary value of protein-free—to the total protein of—. Sure, Barnett .....	373
Muller, John Hughes, and Iszard, Miriam Stewart. The effects of germanium dioxide upon the blood.....	181
Non-protein nitrogen and blood pressure in relation to kidney and heart lesions. Floyd, Rolfe.....	759

## INDEX - Volume 3—*Continued*

Nutrition. On the relations between fertility and—. III. The normal reproductive performance of the rat. Evans, Herbert MacLean, and Bishop, Katharine Scott.....	201
——— Amino-acids in—. VI. The nature of the supplementary value of protein-free milk to the total proteins of milk. Sure, Barnett.....	373
——— VII. Further studies on the cause of the nutritive inadequacy of the proteins of the Georgia velvet bean ( <i>Stizilobium Deeringianum</i> ). Sure, Barnett.....	383
O'Kelly, R. E., and Sure, Barnett. See Sure, Barnett, and O'Kelly. Paton, Julia B., and others. Effect of purification of casein on its food value .....	1
Phlorizin Glycosuria and diabetes. Allen, Frederick M.....	623
Phosphates, the significance of—in the production of tetany. Underhill, Frank B.; Gross, Erwin G., and Cohen, William.....	679
Rations. Effect of various—on young normal guinea pigs and on young guinea pigs inoculated with tuberculosis. Davis, Marguerite .....	725
——— Effect of—containing whole and skimmed milk on young growing puppies. Davis, Marguerite.....	711
Renner, Dan S., and others. Fasting and diets in treatment of epilepsy .....	317
Saccharin. Studies on the physiological action of—. Carlson, A. J.; Eldridge, C. J.; Martin, H. P., and Foran, F. L.....	451
Sansum, W. D.; Blatherwick, N. R.; Smith, Florence H.; Long, M. Louisa; Maxwell, L. C.; Hill, Elsie; McCarty, Ray, and Cryst, J. H. The treatment of diabetes with insulin. A report of the methods followed and the results obtained in the first one hundred cases .....	641
Scott, D. A., and Best, C. H. Possible sources of insulin.....	177
Sherrill, James W. Clinical observations with insulin. 2. The influence of carbohydrate and protein on diabetes and insulin requirement .....	13
Sherrill, James W., and Allen, Frederick M. Diet treatment of diabetes insipidus .....	479

## INDEX - Volume 3—*Continued*

Smith, Florence H., and others. Treatment of diabetes with insulin	641
"Spring" Cod Liver Oil. The vitamine potency of—. Holmes, Arthur D. ....	393
Sterility. The production of—with nutritional regimes adequate for growth and its cure with other foodstuffs. Evans, Herbert MacLean, and Bishop, Katharine Scott.....	233
Sure, Barnett. Amino-acids in nutrition. VI. The nature of the supplementary value of protein-free milk to the total proteins of milk .....	373
——— Amino-acids in nutrition. VII. Further studies on the cause of the nutritive inadequacy of the proteins of the Georgia velvet bean ( <i>Stilzlobium Deeringianum</i> ).....	383
——— and O'Kelly, R. E. The distribution of sulfur in protein-free milk .....	365
Underhill, Frank P.; Gross, Erwin G., and Cohen, William. Studies in inorganic metabolism. Study III. The significance of phosphates in the production of tetany.....	679
Weeks, David F.; Renner, Dan S.; Allen, Frederick M., and Wishart, Mary B. Observations on fasting and diets in the treatment of epilepsy .....	317
Wishart, Mary B. See Weeks.....	317



## THE EFFECT OF PURIFICATION OF CASEIN ON ITS FOOD VALUE.

By CASIMIR FUNK, JULIA B. PATON AND LOUIS FREEDMAN.

*From the Biochemical Laboratory, College of Physicians and Surgeons,  
Columbia University and the Research Laboratory  
of H. A. Metz, New York City.*

One of the writers emphasized in 1912<sup>1</sup> that crude casein and other products obtained from milk, when not properly purified, retain small quantities of vitamins. Consequently, Funk and Macallum<sup>2</sup> subjected casein to a prolonged and repeated treatment with boiling alcohol, prior to its incorporation into an artificially compounded diet for rats. Later investigators have used other methods of casein purification, probably equally efficient in ridding this protein of the adhering vitamins.

Since the work of Mueller,<sup>3</sup> who found that certain proteins, notably casein, contain one or more substances which promote the growth of bacteria of the streptococcus type, it has seemed possible that certain protein materials may carry down with them vitamin-like substances, which do not belong to any of the hitherto recognized types.

Freedman and Funk<sup>4</sup> were able to demonstrate on a series of carefully purified plant and animal proteins, that the accepted methods of purification of proteins do not suffice to eliminate the factor responsible for the growth of the streptococcus, as the absorptive affinity of the precipitating protein appears to be exceedingly marked.

It was thought, therefore, that by using crude casein, a protein which can be brought into solution in the form of its sodium salt, and treating the resulting solution with an agent which displays a greater absorptive power than the protein itself, the nutritive factor we were seeking could be successfully separated and its properties studied.

By using this method of purification we were indeed able to prove that by shaking a solution of sodium caseinate with either fuller's earth or norite, we had removed from the casein the growth-promoting factor for bacteria and that the recovered casein was entirely inactive in this respect.<sup>4</sup> On the other hand we were able to recover this factor from both fuller's earth and

norite. The method used can be applied to every protein or other material which can be dissolved, for the purpose of eliminating vitamins of the water-soluble type. By adding an oxidizing agent during the shaking with the absorbent, we thought that we could prepare casein and other suitable food materials free from water-soluble and also A vitamins.

The food value of casein purified in this manner interested us from two points of view. First, having this supposedly vitamin-free casein we wished to ascertain whether or not such protein had suffered other changes which had rendered it unsuitable for feeding purposes; in short, whether a diet made up of such casein supplemented in a suitable way would again be a complete diet for rats. Second, we were anxious to know whether such purified and vitamin-free casein would throw any light on the etiology of pellagra and sprue.

Ever since the report of the British Pellagra Commission<sup>5</sup> concerning the Turkish prisoners of war in Egypt, pellagra has been attributed to a deficiency in certain aminoacids, rather than a deficiency in a vitamin, because this commission expressed a definite conclusion that lack of tryptophane was responsible for the outbreak of pellagra. The conclusion was also reached that if the biological value of the ingested proteins falls below an equivalent of 40 gm. casein per day a definite danger of pellagra exists. The old biological values for the proteins, however, are as Mitchell<sup>6</sup> quite rightly points out, too high for milk and too low for cereals, so that the above stated protein equivalent will probably necessitate a correction. Goldberger<sup>7</sup> some time ago made the observation that pellagra outbreaks are particularly numerous in sections where little or not animal protein is consumed. Later Goldberger<sup>8</sup> came to the conclusion that vitamins are practically excluded from the etiology of pellagra, provided we know all of the vitamins. Goldberger and Tanner<sup>9</sup> have tried the therapeutic effect of cystine and tryptophane and believe that they have obtained encouraging results. Finally the same authors<sup>10</sup> have recently seen recurrences of pellagra on a diet adequate in respect to known vitamins and necessary salts but containing only corn, peas, potatoes and vegetables as the source of protein. It seems unwise, at the present stage of our limited knowledge, to draw a sharp distinction between the food value of plant and animal proteins. While some proteins of plant origin lack one or more of the necessary aminoacids, we possess



no reliable data on these plant products as they exist in combinations in our foods. Furthermore such an assumption does not seem justified if one considers the dependence of the animal world on plants, the perfect nutritive state of a number of strictly herbivorous animals, unless we assume that the latter possess unusual synthetic powers. Goldberger and Tanner describe one case of pellagra which developed on a diet containing 24 gm. of milk proteins a day and which was cured by further addition of 45 gm. of milk proteins to an otherwise unchanged diet. While this well observed case merits our attention, it cannot, in our opinion, form a basis for an unbiased conclusion as to the etiology of pellagra due to lack of animal protein since other factors besides milk protein were added at the same time. Their argument is further weakened by the occurrence, rather rare it is true, of infantile pellagra in breast-fed infants, of which three more cases have been brought recently to our attention by Lustberg and Birchett.<sup>11</sup> Here it is unlikely that the food lacks essential amino acids, and Goldberger explains the latter cases, as well as the case in an adult, described above, by characteristic differences in the requirements of various individuals as regards animal protein. The work on the food value of cereal and potato proteins by Hindhede<sup>12</sup> and others who observed an excellent nutritional state in individuals having about 20 gm. of assimilated potato or cereal protein, and the non-existence of pellagra in populations which exist almost exclusively on potatoes, cabbage and coarse bread, speaks very definitely against the conception of Goldberger and his school that lack of amino acids is the primary cause of pellagra. These facts together with others which we know about pellagra are not at all incompatible with the idea that pellagra may be due to deficiency of a yet unknown vitamine. This is a possibility which Goldberger does not deny but regards as extremely unlikely. But then it is very probable that we are far from knowing all the types of vitamins which may play a rôle in our nutrition. When we consider the uncertainty as to the etiology of pellagra we must feel somewhat surprised that considering the high stand of the science of nutrition, *we do not as yet know with certainty what type of protein and how much of it is necessary for our nutritive wants.* It seems that in our modern researches in nutrition we are losing ourselves in a mass of detail, but are *neglecting the fundamental facts about protein requirements.* The sooner this gap is filled, the better.

On the other hand the idea that many dietary deficiencies may be corrected by a liberal milk diet has some dangers. It is quite likely that the various deficiencies in diets can be corrected in many other ways than by addition of milk. In this connection it is interesting to note the results of Pucher and Cori,<sup>13</sup> where a rather definite instance of malnutrition in cats, with an appearance of a sugar-like substance in the urine was noticed after the inclusion of a fair proportion of milk in their diet. Webb-Johnson<sup>14</sup> is of the opinion that a milk diet is one of the commonest causes of pyorrhea and decay of teeth. This latter assertion, however, awaits experimental verification.

As regards sprue, Elders<sup>15</sup> asserts that it is a deficiency disease, and possibly the clearing of the etiology of pellagra will help us find the cause of sprue. Elders believes that even the severest forms can be cured by dietetic treatment without the help of any drugs.

Keeping in mind the possibility of a new vitamine-like substance which might play a rôle in preventing pellagra, we made two separate experiments on several groups of rats. The casein that enters this diet was so treated that it was presumably devoid, not only of known vitamines but also of the substance adhering to certain protein materials which influences the growth of streptococci.<sup>4</sup> With this casein we were able to produce a definite condition of malnutrition in rats although it probably has no analogies with pellagra. It is, however, interesting to note that we were unable to supplement effectively this diet either with the known vitamines, with the fuller's earth itself or by extracts made from fuller's earth. Casein purified according to our method did not differ in chemical behavior from the ordinary casein, and the only reason for our inability to supplement it consists perhaps in the formation of a disturbing factor by the absorbing agent which we employed. It was our intention to extend the experiments to dogs and also to test the food value casein treated with hydrogen peroxide alone and not with fuller's earth. We are unable at present, however, to continue the work planned and we think perhaps the preliminary results are sufficiently interesting to make them known in their present form.

#### *Experimental.*

With the purified casein we have made experiments on two series of animals. We are unable to continue the work for the



present but we are planning to repeat it on rats and on other animals which show perhaps more characteristic symptoms of malnutrition.

*The preparation of casein.*—The method was essentially as follows: 300 gm. of technical casein was suspended in 3 litres of 1 per cent. hydrogen peroxide, and 90 cc. of 5 per cent. NaOH was added until solution became neutral. Shaken with 167 gm. of English fuller's earth for 2 hours. The mixture was allowed to settle down and the supernatant fluid siphoned off and filtered, the sediment centrifuged, washed and united with the main filtrate. The casein was recovered from the sodium caseinate solution by precipitation with dilute acetic acid. Yield 180 gm. The casein used in experiment II was not treated with hydrogen peroxide.

*Treatment of activated fuller's earth.*—The amount dry was 183 gm., an increase of 16 gm. over the amount originally used, or approximately 10 per cent. An analysis by Kjeldahl showed 2.85 per cent. of nitrogen (large part of which was adhering casein). Analysis of the original fuller's earth, representing about 450 gm. of casein, was shaken for a few with casein solution caused an increase in nitrogen of 2.75 per cent.

*Preparation of extracts of fuller's earth.*—150 gm. of the activated fuller's earth, representing about 450 gm. of casein, was shaken for a few minutes with 1 litre of 1 per cent. baryta solution of 50° C. The solution was quickly filtered on a large Buchner funnel and the filtrate immediately neutralized with sulfuric acid. The filtrate was evaporated *in vacuo* to 125 cc., so that 1 cc. of the extract represented 3.6 gm. of casein or, as we shall see later, 20 gm. of the total food mixture given to the rats. An analysis of nitrogen by Kjeldahl showed that the total extract contained 0.28 gm. of nitrogen, representing about 20 per cent. of the total nitrogen of the activated fuller's earth itself.

#### *Analysis of Various Casein Samples.*

For the nitrogen determination the various samples were dried at 100° until constant in weight.

I. Technical casein .....	12.52% N
Purified with fuller's earth.....	13.5
II. Technical casein .....	12.5
Purified with fuller's earth.....	13.21
Casein purified by several re-precipitations...	12.72
Harris vitamine-free casein.....	13.24

All our nitrogen analyses of casein were very much below the accepted figures, probably due to the mode of preparations or other unknown reasons. However, the nitrogen was higher as compared with the original product and the preparation was much cleaner looking. Both the untreated and the purified samples of casein were subjected to a series of protein color tests, characteristic for several of the aminoacids, and no differences were observed except that our purified casein gave clearer color tests.

*Experiment I.**Casein treated with hydrogen peroxide and fuller's earth.*

In this series of experiment six groups of rats were used, five rats in each group of the same litter, the basal diet being as follows:

Casein 18%, starch 54%, Crisco 24%, salts 4%.

Aside from this, 1 mg. of butter, 66 mg. of Harris B vitamine, and 2 cc. (later 1 cc.) of orange juice were given separately from the food. The groups were arranged as follows:

	Males	Females
I. Purified casein and no vitamins.....	5	
II. " " plus A and B.....	2	3
III. " " " A, B and C.....	3	2
IV. " " " A, B, C and activated.....		
V. Purified casein 15 gm. plus 3 gm. of Harris fuller's earth.....	2	3
(vitamine B-free) casein .....	5	
VI. Harris casein and no vitamins.....	5	

*Chart I, Experiment I.*

*Composite curves of two series of five rats each.*

Series V Purified Casein 18% and

Harris Casein 3%, no vitamine.

Series II Purified Casein + A and B.



TABLE I. 22 23 24 1 5 8 12 15 19 22 26 29 2 5 9 12 16 19 23 26 30 2 6 9 13 16 20 23 27 30 4 7 11 14

In summarizing some of the results of this experiment, we may state that in three groups which had the vitamine-free food, no essential difference was noticed whether our purified casein or Harris B-free casein was given, or when a small proportion of Harris casein was added to casein subjected to fuller's earth treatment. Occasional addition of activated fuller's earth or extracts did not save the animals. They showed no growth and died early in experiment.

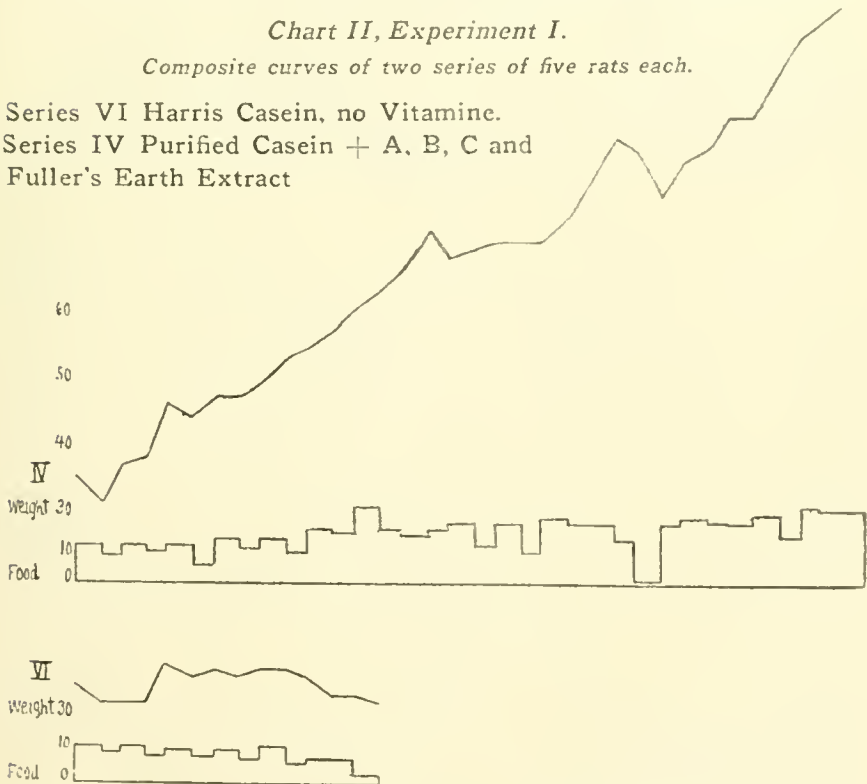
In the series II (Chart I) which had an addition of vitamins B and A, growth proceeded at a slow rate, very much behind the normal curve. The addition of vitamine C, which was made for the purpose of studying whether orange juice could supply something eliminated from casein, made perhaps a slight difference in the rate of growth, but the effect was of small magnitude. In the

*Chart II, Experiment I.*

*Composite curves of two series of five rats each.*

Series VI Harris Casein, no Vitamine.

Series IV Purified Casein + A, B, C and  
Fuller's Earth Extract



3. 2 17 21 21 28 1 8 12 13 19 22 26 29 2 5 9 12 16 19 23 26 30 2 6 9 13 16 20 23 27 30 4 7 11 14

group IV (Chart II) which received in addition to the vitamins, activated fuller's earth, or extracts made from it (1 cc. = 3.6 gm. of casein), the effect was far from beneficial, these animals being behind in growth as compared with those receiving only vitamins in addition. With activated fuller's earth the effects were particularly disastrous. This addition caused in both series of experiments an almost total loss of fur, and the hair appeared after one week of cessation of its administration. Untreated fuller's earth did not have, apparently, the same bad effect.

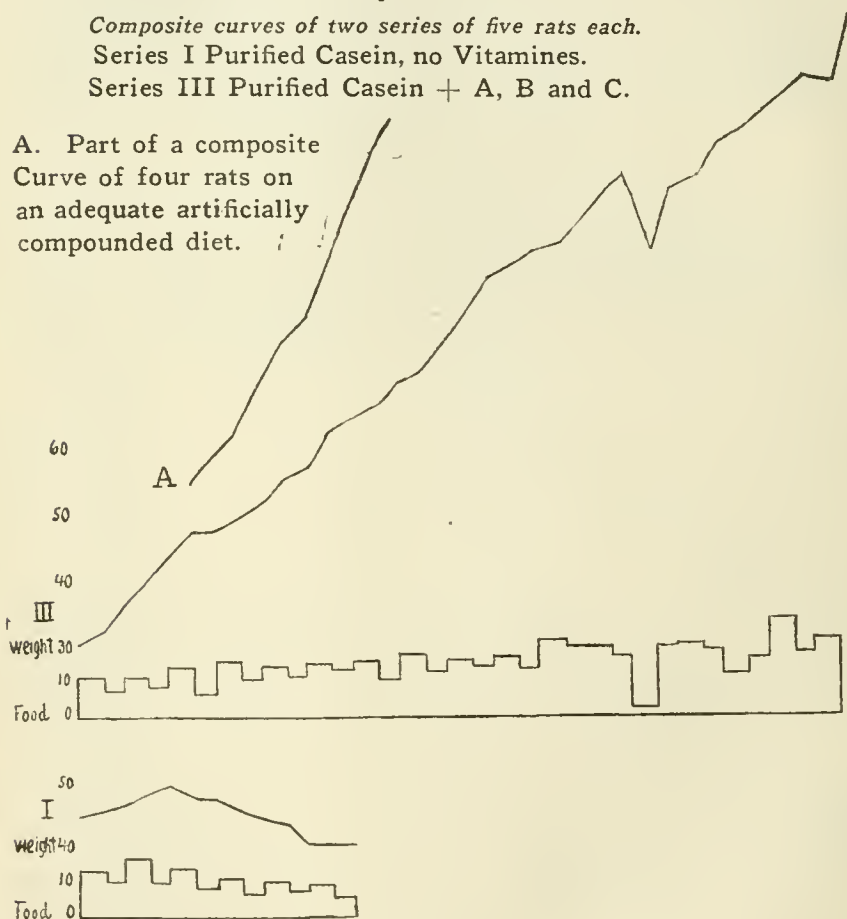
### Chart III, Experiment I.

Composite curves of two series of five rats each.

Series I Purified Casein, no Vitamines.

Series III Purified Casein + A, B and C.

A. Part of a composite Curve of four rats on an adequate artificially compounded diet.



Summarizing once more the results of these series, we may be permitted to draw the conclusion that the treatment of crude casein with fuller's earth has impaired greatly the nutritive value of this protein material. We were unable, however, to find a dietary supplement, which would offset the effect of such a treatment. That the so-treated casein has lost much of its nutritive value is shown by the results of the next series of experiments, where the value of our purified casein is compared with crude casein and Harris casein without the addition of vitamins.

## II. Experiment.

*Casein treated with fuller's earth and oxidized by heating in air.*

### Chart IV, Experiment II.

*Composite curves of four series of four rats each.*

Series I Crude casein.

Series II Harris casein.

Series III Oxidized Purified Casein.

Series IV Oxidized Purified Casein + Fuller's Earth.



In this series four groups of rats were taken, four rats in each group (two males and two females). Without the addition of vitamins the experiment\* lasted much shorter and was planned as follows:

- I. Crude commercial casein.
- II. Harris casein.†
- III. Purified casein with fuller's earth, not treated with hydrogen peroxide, but oxidized at 100° in shallow dishes.
- IV. Purified casein plus fuller's earth.

Here on crude and Harris casein there was maintenance lasting for about 20 days, followed by decline; whereas with purified casein, according to our method, a rapid decline set in immediately, which was not offset by administration of fuller's earth. An addition of vitamins B and A resulted in immediate improvement. (See Chart IV where arrow is placed.)

\* Miss Olive Sheets helped us with this experiment and we wish here to extend our thanks to her.

† The Harris casein was one of the earlier preparations put out by the Harris Laboratories and probably was not entirely vitamin-free.

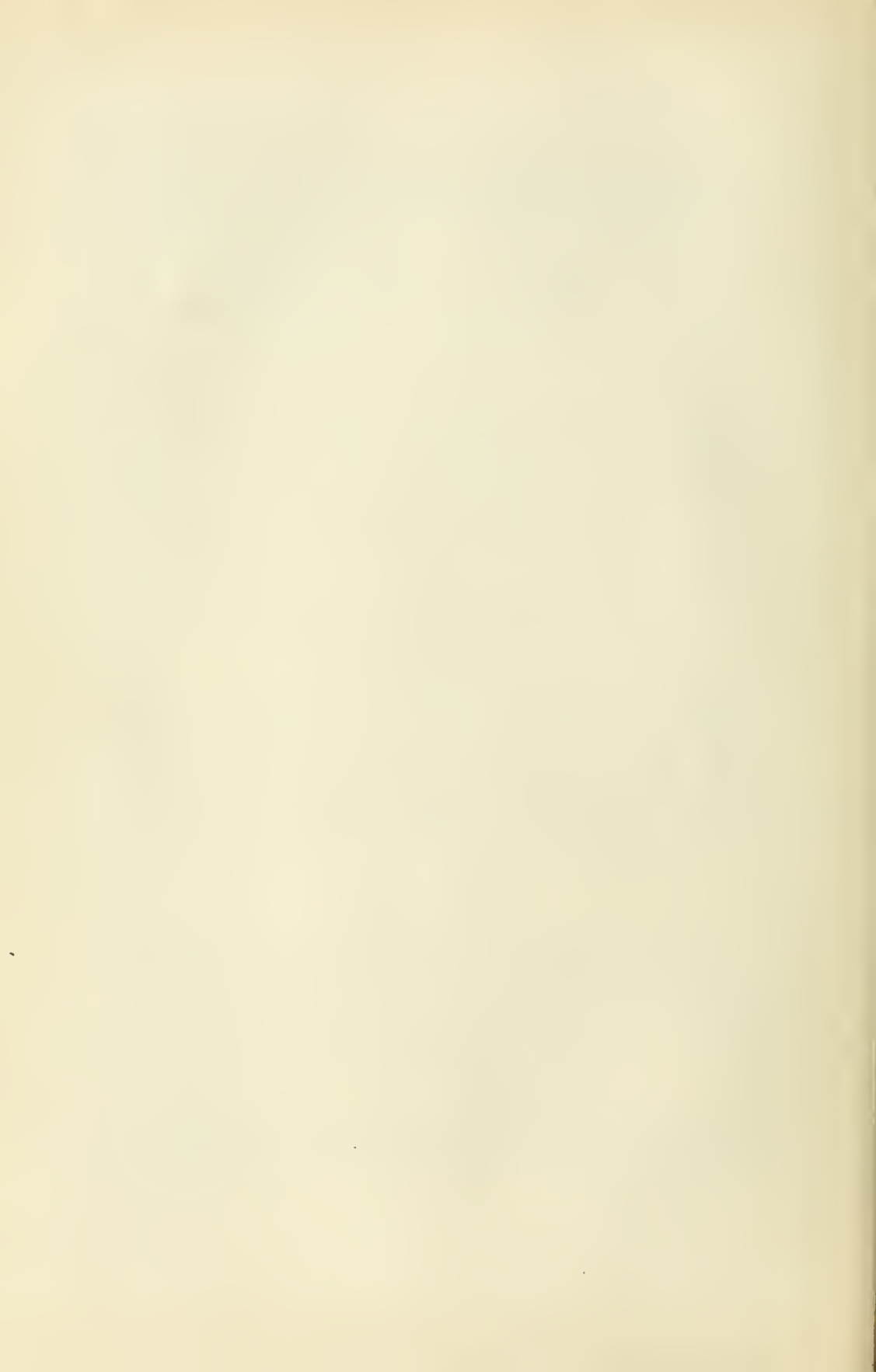
### Summary.

Casein in the form of neutral sodium caseinate which had undergone a treatment with fuller's earth and had been oxidized either by hydrogen peroxide or by heating in air, lost considerably in nutritive value for rats. Such casein has been found devoid of a substance that stimulates the growth of streptococci. So far we have been unable to correct this deficiency by the addition of the activated fuller's earth obtained from the casein.

### REFERENCES.

1. Funk, Casimir, *J. State Med.*, June, 1912.
2. Funk, Casimir, and Macallum, A. B., *J. Biol. Chem.*, 23, 1915, 413.
3. Mueller, J. H., Comm. I and II, *J. Bact.*, 7, 1922, 309, 325.
4. Freedman, L., and Funk, Casimir, *J. Metabol. Res.*, 1, 1922, 457, 469.
5. Port Said Publ. Health Dep., Egypt, 1916. *Rep. Brit. Med. Res. Comm.* No. 38, 1919.
6. Mitchell, H. H., *Amer. J. Publ. Health*, 13, 1923, 17.
7. Goldberger, J., and Wheeler, G. A., *Hyg. Labor. Bull.*, Wash. 120, 1920, 7.
8. Goldberger, J., *J. Amer. Med. Assn.*, 78, 1922, 1676.
9. Goldberger, J., and Tanner, W. F., *Publ. Health Rep. No.* 732, 1922.
10. Goldberger, J., and Tanner, W. F., *J. Amer. Med. Assn.*, 79, 1922, 2132.

11. Lustberg, S. R., and Birchett, J. A. K., *Arch. Ped.*, 39, 1922, 255.
12. Hindhede, M., Work reviewed in *Vitamines*, by Casimir Funk, II Edit., Williams & Wilkins, 1922.
13. Pucher G. W., and Cori, K. F., *J. Biol. Chem.*, 54, 1922, 567.
14. Webb-Johnson, C., *Diet for Women*. Mills & Boon.
15. Elders, C. *Indische Spruw* (Pamphlet); *Ned. Tijdrs. v. Gen.* 61, 1917, 1253; *Ibid.* No. 21, 1920; *Ibid.* No. 21, 1922.





## CLINICAL OBSERVATIONS WITH INSULIN.

### 2. *The Influence of Carbohydrate and Protein on Diabetes and the Insulin Requirement.*

By JAMES W. SHERRILL, M.D.

*The Physiatrie Institute, Morristown, New Jersey.*

This paper presents a series of experiments concerning the insulin requirement of diabetic patients as modified by additions, withdrawals and substitutions of foods, particularly carbohydrate and protein. Information on this subject is evidently important for the practical management of the insulin treatment and for the theoretical problem of the physiological or chemical function of insulin. The histories of these patients are given in paper No. 1, in the preceding number of this journal. The methods used were the same as described in previous publications from this Institute, and the protocols are mostly self-explanatory.

#### REMARKS ON TABLE 1.

This man was admitted Jan. 18, 1923, with superficial gangrene of the 5th toe of the right foot. Heavy glycosuria and hyperglycemia of 0.334 per cent. were present. He was treated without insulin for two weeks, during which time glycosuria was abolished and the plasma sugar reduced below 0.2 per cent. on a diet of only 40 gm. protein and 5 gm. carbohydrate. When the diet was increased only to 50 gm. protein, 10 gm. carbohydrate and 800 calories, the plasma sugar promptly rose over 0.2 per cent. Beginning Feb. 4, 2 units of insulin were given daily, with only slight reduction in hyperglycemia. On Feb. 9, the dosage was increased to 4 units per day, which reduced the plasma sugar, but not to normal. The difference in the demand on the pancreatic function represented by hyperglycemia and by normal plasma sugar can thus be estimated in terms of insulin as usual.

An addition of 100 gm. carbohydrate with its 400 calories, beginning Feb. 18, caused glycosuria within 24 hours, which was not stopped until the insulin was increased to 12 units per day. Hyperglycemia persisted with this dosage.

The experiment shows merely that in this case 8 units of insulin seemed to be required for the assimilation of the added 100 gm. of carbohydrate.

#### REMARKS ON TABLE 2.

This girl's regular diet during about 3 years at home had been 50 gm. protein, 10 gm. carbohydrate and 1000 calories. At this admission, Jan. 17, she was free from glycosuria, but had moderate hyperglycemia of 0.178 per cent. For the first few days after admission she received the

diet mentioned with 4 units of insulin daily. This dosage reduced the plasma sugar to the nearly normal value of 0.143 per cent. The difference between hyperglycemia and normal plasma sugar is thus again illustrated.

TABLE 1  
Case No. 1316

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Jan.								
28	50	62	10	800	0	...	...	...
29	"	"	"	"	0	...	115	...
30	"	"	"	"	0	223	...	...
31	"	"	"	"	0	...	115	...
Feb.								
1	"	"	"	"	0	...	...	...
2	"	"	"	"	0	234	115	...
3	"	"	"	"	0	...	...	...
4	"	"	"	"	0	...	113	2
5	"	"	"	"	0	...	...	2
6	"	"	"	"	0	...	113	2
7	"	"	"	"	0	209	...	2
8	"	"	"	"	0	...	112	2
9	"	"	"	"	0	...	...	4
10	"	"	"	"	0	...	112	4
11	"	"	"	"	0	171	...	4
12	"	"	"	"	0	...	113	4
13	"	"	"	"	0	...	...	4
14	"	"	"	"	0	166	113	4
15	"	"	"	"	0	...	...	4
16	"	"	"	"	0	...	113	4
17	"	"	"	"	0	...	...	4
18	50	62	110	1200	+	...	114	4
19	"	"	"	"	+	220	...	4
20	"	"	"	"	+	...	115	4
21	"	"	"	"	+++	...	...	8
22	"	"	"	"	+	...	113	8
23	"	"	"	"	+	...	...	8
24	"	"	"	"	+	258	113	8
25	"	"	"	"	+	...	...	8
26	"	"	"	"	+	...	113	8
27	"	"	"	"	+	230	...	12
28	"	"	"	"	+	...	112	12
Mar.								
1	"	"	"	"	+	230	...	12
2	"	"	"	"	0	...	112	12
3	"	"	"	"	0	...	...	12
4	"	"	"	"	0	...	113	12
5	"	"	"	"	0	...	...	12
6	"	"	"	"	0	169	112	12
7	"	"	"	"	0	...	...	12
8	"	"	"	"	0	209	113	12
9	"	"	"	"	0	...	...	12
10	"	"	"	"	0	211	112	12

Jan. 21, the diet was increased to 50 gm. protein, 20 gm. carbohydrate and 1300 calories, and the insulin to 6 units daily. Glycosuria did not occur, but slight hyperglycemia continued.

TABLE 2  
Case No. 529

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Jan.								
17	50	84	10	1000	0	178	44	4
18	"	"	"	"	0	162		4
19	"	"	"	"	0	...	43	4
20	"	"	"	"	0	...		4
21	50	113	20	1300	0	143	44	6
22	"	"	"	"	0	...		6
23	"	"	"	"	0	...	46	6
24	"	"	"	"	0	138		6
25	"	"	"	"	0	...	45	6
26	"	"	"	"	0	151		6
27	"	"	"	"	0	...	46	6
28	50	113	70	1500	0	...		6
29	"	"	"	"	0	182	46	6
30	"	"	"	"	0	...		6
31	"	"	"	"	0	223	47	6
Feb.								
1	50	113	100	1620	0	...		6
2	"	"	"	"	0	246	47	6
3	"	"	"	"	0	...		6
4	"	"	"	"	2.99	...	47½	6
5	"	"	"	"	15.84	268		6
6	"	"	"	"	13.14	...	48	8
7	"	"	"	"	0	300 366*		10
8	"	"	"	"	12.25	...	48	10
9	"	"	"	"	3.20	319		12
10	"	"	"	"	0	258 156*	49	15
11	"	"	"	"	0	230 140†	49	15
12	100	100	100	1700	0	272		15
13	"	"	"	"	2.64	100*	50	15
14	"	"	"	"	+	246 131*	50	15(A)
15	"	"	"	"	+	312 159*		15
16	"	"	"	"	4.81	283 226*	50	15
17	"	"	"	"	4.80	...	51	15

(A) Insulin changed from t.i.d. to b.i.d.

\*Plasma sugar at 7 p.m.

†Plasma sugar at 1 p.m.

Beginning Jan. 28, the effect of the addition of carbohydrate with added calories was studied. The addition of 50 gm. daily, Jan. 28 to 31, caused

TABLE 3  
Case No. 1238

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Dec. 31	25	23	10	350	++++	577	...	4*
Jan. 1	"	"	"	"	++++	...	24	4
2	"	"	"	"	++++	...	...	4
3	"	"	"	"	++++	...	21	4
4	25	14	10	266	++++	...	...	4
5	"	"	"	"	++++	...	25	4
6	"	"	"	"	++++	...	...	4
7	"	"	"	"	++++	...	26	4
8	"	"	"	"	++++	...	...	4
9	"	"	"	"	++++	...	22	4
10	"	"	"	"	++++	455	...	4
11	"	"	"	"	7.50	...	24	4
12	"	"	"	"	7.84	...	...	4
13	"	"	"	"	5.25	...	22	4
14	"	"	"	"	2.80	...	...	6
15	"	"	"	"	1.15	...	23	6
16	"	"	"	"	0	...	...	6
17	"	"	"	"	2.90	...	23	6
18	"	"	"	"	.87	429	...	9
19	"	"	"	"	+	...	24	9
20	"	"	"	"	+	...	...	9
21	"	"	"	"	0	294	23	9†
22	"	"	"	"	0	...	...	9
23	"	"	"	"	0	...	23	9
24	"	"	"	"	0	230	...	9
25	25	31	5	400	0	...	23	12
26	"	"	"	"	0	189	...	12
27	"	"	"	"	0	...	23	12
28	"	"	"	"	0	...	...	12
29	40	44	10	600	0	...	23	12
30	"	"	"	"	0	...	...	12
31	"	"	"	"	0	234	23	12
Feb. 1	"	"	"	"	0	...	...	12
2	"	"	"	"	0	...	23	12
3	"	"	"	"	0	...	...	12
4	"	"	"	"	0	...	23	12
5	"	"	"	"	0	211	...	12
6	"	"	"	"	0	...	23	12
7	"	"	"	"	0	...	...	12
8	"	"	"	"	0	...	23	12
9	40	44	75	856	0	254	...	12
10	"	"	"	"	2.20	...	24	12
11	"	"	"	"	3.50	...	...	12
12	"	"	"	"	8.12	...	23	12
13	"	"	"	"	12.2	...	...	12
14	"	"	"	"	6.0	357	24	12
15	"	"	"	"	+	...	...	12
16	"	"	"	"	+	...	23	18

\*Extract given b.d.

†Extract given t.i.d.

TABLE 3.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.								
17	40	44	75	856	2.99	...	...	18
18	"	"	"	"	+	...	24	18
19	"	"	"	"	5.75	211	...	18
20	"	"	"	"	+	...	24	18
21	"	"	"	"	0	...	...	20
22	"	"	"	"	0	...	25	20
23	"	"	"	"	0	...	...	20
24	"	"	"	"	0	173	25	20
25	60	35	75	856	0	...	...	20
26	"	"	"	"	0	...	25	20
27	"	"	"	"	0	...	...	20
28	"	"	"	"	0	...	25	20
Mar.								
1	"	"	"	"	0	189	...	20
2	"	"	"	"	0	...	...	...
3	"	"	"	"	0	...	...	...
4	"	"	"	"	0	199	26	20
5	"	"	"	"	0	209	...	20
6	"	"	"	"	0	...	26	20
7	"	"	"	"	0	...	...	20
8	"	"	"	"	1.41	...	27	20
9	"	"	"	"	0	...	...	20
10	"	"	"	"	0	...	27	20
11	"	"	"	"	0	...	...	20

a rapid rise of plasma sugar to 0.223 per cent. Feb. 1, the carbohydrate was further increased by 30 gm., making 100 gm. total carbohydrate and 1620 total calories. Hyperglycemia increased and glycosuria began Feb. 4. It was stopped only by an increase of insulin dosage to 15 units. In this instance, therefore, a total addition of 80 gm. carbohydrate seemed to create an extra requirement of 9 units of insulin per day.

Feb. 12, the protein was increased to 100 gm., and the fat was slightly reduced, so that the total calories were raised only to 1700. Slight glycosuria resulted, but not above 4.81 gm. The experiment had to be interrupted at this point.

#### REMARKS ON TABLE 3.

On a diet of 25 gm. protein, 10 gm. carbohydrate and 350 calories, this child showed glycosuria until the insulin dosage was increased to 9 units per day. Hyperglycemia still persisted.

After a slight change to a diet of 400 calories on Jan. 25, on Jan. 29 both protein and fat were increased, making the diet 40 gm. protein, 10 gm. carbohydrate and 600 calories. A distinct rise of plasma sugar resulted, but no glycosuria.

Feb. 9, the carbohydrate was increased to 75 gm., and as the protein and fat were left unchanged, the total calories were thus raised to 856. The

resulting glycosuria was barely halted by an increase of insulin to 20 units daily. Hyperglycemia was still present. The experiment indicates that in this patient 8 additional units of insulin were required for the assimilation of the 65 gm. of added carbohydrate. The duration of the experimental period was sufficient to make the result quite definite.

TABLE 4  
Case No. 1303

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Jan.								
17	25	10	5	210	2.88	375	43	..
18	"	"	"	"	2.24	...	...	..
19	"	"	"	"	—	...	45	..
20	"	"	"	"	+	...	...	4*
21	"	"	"	"	0	...	44	4
22	"	"	"	"	0	...	...	4
23	"	"	"	"	0	180	43	4
24	"	"	"	"	0	...	...	4
25	"	"	"	"	0	199	43	4
26-28	"	"	"	"	0	...	40	4
29	30	11	55	439	0	136	43	4
30-31	"	"	"	"	0	...	43	4
Feb.								
1	30	11	110	659	2.41	214	...	4
2	"	"	"	"	0	...	41	4
3	"	"	"	"	7.40	...	...	4
4	"	"	"	"	9.75	283	42	4
5	"	"	"	"	6.72	...	...	4
6	"	"	"	"	4.90	...	42	4
7	"	"	"	"	+	272	...	8
8	"	"	"	"	0	...	42	8
9	"	"	"	"	0	...	...	8
10	"	"	"	"	5.25	...	42	8
11	60	51	75	1000	+	234	...	8
12	"	"	"	"	0	...	42	8
13	"	"	"	"	0	...	...	8
14	"	"	"	"	0	200	...	8

\*Insulin given b.d.

#### REMARKS ON TABLE 4.

This patient was received on Jan. 4, 1923, with heavy glycosuria and moderate acetonuria. The case was one of severe diabetes. From Jan. 4 to 20, the urine constantly contained sugar, although the diet was less than 300 calories per day. The plasma sugar ranged from 0.334 to 0.375 per cent. The body weight on admission was 40 lb., but it increased to 45 lb. by Jan. 20, on account of slight edema.

Four units of insulin were given daily, beginning Jan. 20. The urine became sugar-free within 24 hours, and the plasma sugar decreased. The

boy remained thin and weak, and on two occasions showed signs of hypoglycemia. By Jan. 28 the plasma sugar before breakfast showed a low value of 0.136 per cent.

From Jan. 29 to Feb. 1, an addition of 50 gm. of carbohydrate failed to produce glycosuria. A further addition of 55 gm. on Feb. 1 produced glycosuria immediately. On Feb. 1, 2.41 gm. of dextrose was excreted, and increased gradually to 9.75 gm. by Jan. 4, while the insulin dosage was kept unchanged at 4 units per day. Beginning Jan. 7, the insulin dosage was increased to 8 units per day. The urine was free from sugar on Jan. 8 and 9, but on Jan. 10 contained 5.25 gm. of glucose.

In this case an addition of 105 gm. carbohydrate, with the extra calories, seemed to require approximately 4 additional units of insulin. The possibility of error in this reckoning due to gain in the patient's tolerance is not excluded.

#### REMARKS ON TABLE 5.

The admission of this patient in coma, and the high insulin dosage required on this account on Jan. 19 and 21, were described in paper No. 1.

Both the diet and insulin dosage were then adjusted to suit the needs after the acidosis had cleared up. Feb. 9 to 14, the diet was 60 gm. protein, 40 gm. carbohydrate and 2479 calories. Dosage of 36 units of insulin per day tended to produce evening hypoglycemia. On Feb. 10, the evening analysis of 0.086 per cent. probably did not represent the minimal level to which the blood sugar fell, and there were slight hypoglycemic symptoms. The insulin was then reduced to 27 units daily, which was not quite sufficient, as indicated by the glycosuria, Feb. 12 to 14.

An addition of 100 gm. carbohydrate was then made without change in the other foods, thus raising the total calories to 2879 per day. Increasing glycosuria resulted, the control of which was found to require about 50 units of insulin per day.

The different periods in this experiment were too short for exact results, but if the insulin requirement prior to the carbohydrate addition be estimated at approximately 30 units, the conclusion is possible that the assimilation of the added 100 gm. or 400 calories of carbohydrate required in the neighborhood of 20 units of insulin.

#### REMARKS ON TABLE 6.

On a low calory diet of 30 gm. protein and 5 gm. carbohydrate, this man was free from glycosuria with 2 units of insulin per day, and the plasma sugar was reduced below 0.1 per cent.

The insulin was then raised to 4 units, and the diet increased especially in fat, so that by Jan. 31 it amounted to 30 gm. protein, 5 gm. carbohydrate and 2000 calories. The plasma sugar rose to 0.238 per cent. on Feb. 7, and glycosuria would probably have resulted if the experiment had been continued long enough.

Feb. 9, the protein was increased to 200 gm., while fat was reduced so as to keep the total calories constant at 2000. Heavy glycosuria resulted







7	60	231	40	2479	0	0	80	103	36
8	"	"	"	"	0	0	234	103	36
9	"	"	"	2479	0	0	139	101	36
10	"	"	"	"	0	0	"	"	36
11	"	"	"	"	8.40	8.40	"	103	27
12	"	"	"	"	+	+	"	"	27
13	"	"	"	"	7.56	7.56	230	103	27
14	"	"	"	"	+	+	"	"	27
15	60	221	140	2789	2.36	2.36	319	"	27
16	"	"	"	"	+	+	"	"	27
17	"	"	"	"	41.23	41.23	"	102	27
18	"	"	"	"	+	+	"	"	27
19	"	"	"	"	+	+	395	104	27
20	"	"	"	"	+	+	"	"	30
21	"	"	"	"	30.51	30.51	375	104	30
22	"	"	"	"	43.56	43.56	"	"	30
23	"	"	"	"	28.50	28.50	"	107	30
24	"	"	"	"	13.72	13.72	326	107	36
25	"	"	"	"	21.22	21.22	"	"	36
26	"	"	"	"	16.56	16.56	319	107	36
27	"	"	"	"	36.99	36.99	"	"	36
28	"	"	"	"	72.91	72.91	"	107	36
Mar.									
1	"	"	"	"	26.80	26.80	"	107	36
2	"	"	"	"	20.16	20.16	300	107	36
3	"	"	"	"	7.42	7.42	"	"	36
4	"	"	"	"	16.91	16.91	"	107	45
5	"	"	"	"	+	+	306	109	45
6	"	"	"	"	9.62	9.62	"	"	45
7	80	221	120	2789	+	+	312	"	45
8	"	"	"	"	+	+	"	"	50

TABLE 6  
Case No. 755

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan.									
21	30	12	5	228	+	.....	349	111	2*
22	"	"	"	"	0	.....	...	...	2
23	"	"	"	"	0	.....	...	110	2
24	"	"	"	"	0	.....	254	...	2
25	"	"	"	"	0	.....	...	110	2
26	"	"	"	"	0	.....	98	...	2
27	30	84	30	1000	0	.....	81†	104	2
28	"	"	"	"	0	.....	...	...	4
29	"	"	"	"	0	.....	79	103	4
30	"	"	"	"	0	.....	90	...	4
31	30	206	5	2000	0	.....	120‡	104	4
Feb.									
1	"	"	"	"	0	.....	{ 223 173† }	...	4
2	"	"	"	"	0	.....	...	104	4
3	"	"	"	"	0	.....	178‡	...	4
4	"	"	"	"	0	.....	...	100	4
5	"	"	"	"	0	.....	...	...	4
6	"	"	"	"	0	.....	...	105	4
7	"	"	"	"	0	.....	238	...	4
8	"	"	"	"	0	.....	...	105	4
9	200	131	5	2000	16 05	.....	...	...	4
10	"	"	"	"	10.56	.....	...	113	4
11	"	"	"	"	22 08	.....	314	...	4
12	"	"	"	"	28 04	.....	...	110	4
13	"	"	"	"	43 20	.....	357	...	4
14	"	"	"	"	5.12	.....	...	108	8
15	"	"	"	"	+++	.....	...	...	8
16	"	"	"	"	24.30	.....	357	109	8
17	"	"	"	"	4 65	.....	...	...	8
18	"	"	"	"	+++	.....	...	109	12
19	"	"	"	"	+++	.....	385	...	12
20	"	"	"	"	34 90	.....	...	109	12
21	"	"	"	"	0	.....	...	...	16
22	"	"	"	"	0	23.56	143	107	16
23	"	"	"	"	0	26 40	...	...	16
24	"	"	"	"	0	18.90	{ 341 211† }	109	16
25	30	131	100	1700	0	10.43	300	...	16
26	"	"	"	"	0	6.50	...	108	16
27	"	"	"	"	0	4 77	220	...	16
28	"	"	"	"	0	3 91	{ 187‡ }	110	16
Mar.									
1	"	"	"	"	0	4 40	226	...	16
2	"	"	"	"	0	5 49	...	108	16

\*Insulin given b.d.

†Plasma sugar at 11 a.m.

‡Plasma sugar at 7 p.m.

TABLE 6.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Mar.	30	131	100	1700	$\frac{3}{4}$ $\frac{3}{4}$ $\frac{3}{4}$				
3	"	"	"	"	0	4.00	{ 169 127 $\frac{1}{2}$ }	...	16
4	"	"	"	"	0	4.35	199	110	16
5	"	"	"	"	0	3.41	...	...	16
6	"	"	"	"	0	4.06	...	109	16
7	"	"	"	"	0	3.45	{ 156 103 $\frac{1}{2}$ }	...	16
8	"	"	"	"	0	6.08	...	111	16
9	"	"	"	"	0	4.98	...	...	16
10	"	"	"	"	0	5.43	...	111	16
11	30	164	100	2000	0	2.29	...	...	16
12	"	"	"	"	0	3.15	127	111	16
13	"	"	"	"	0	2.78	...	...	16
14	"	"	"	"	0	3.82	...	114	16
15	100	133	100	2000	0	4.30	130	...	16
16	"	"	"	"	0	6.90	...	115	16
17	"	"	"	"	0	6.90	...	...	16
18	"	"	"	"	0	10.22	150	115	16
19	"	"	"	"	0	6.22	...	...	16
20	"	"	"	"	0	7.85	...	115	...
21	"	"	"	"	0	9.38	0.91 $\frac{1}{2}$	...	16
22	"	"	"	"	0	7.93	146	115	16
23	"	"	"	"	0	7.55	...	...	16
24	"	"	"	"	0	10.31	127	116	16
25	100	244	100	3000	0	8.13	152	...	16
26	"	"	"	"	0	...	...	118	16
27	"	"	"	"	0	...	...	...	16
28	"	"	"	"	0	...	200	...	16

‡Plasma sugar at 7 p.m.

immediately, and was stopped only when the insulin was increased to 16 units. The more prompt and powerful glycosuric effect of protein as compared with the caloric equivalent of fat is thus illustrated, but the difference in insulin requirement is not correctly represented by the difference between 4 and 16 units, since the high fat diet would undoubtedly have called for a considerable increase of insulin if it had been continued long enough.

Feb. 25, the protein was reduced to 30 gm. and the carbohydrate increased to 100 gm., so as to keep the theoretical glucose value of the diet practically constant. The total calories were thus reduced to 1700. The plasma sugar steadily fell.

March 11, the fat was increased so as to make 2000 calories, and on March 15 the protein was increased to 100 gm. and fat reduced so as to keep the same 2000 calories. Glycosuria remained absent and the plasma sugar continued close to normal. Here it seemed probable that the

TABLE 7  
Case No. 70

Date	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
1922									
Nov.									
8	30	15	5	275	26.64	7.87	366	117	1
9	"	"	"	"	16.56	7.65	....	....	1
10	"	"	"	"	10.26	9.25	....	118	1
11	"	"	"	"	9.31	4.15	300	....	1
12	"	"	"	"	8.92	4.62	....	116	2
13	"	"	"	"	9.38	4.22	....	....	2
14	"	"	"	"	9.17	4.98	300	116	2
15	"	"	"	"	8.65	9.06	....	....	2
16	"	"	"	"	4.57	4.69	....	120	4
17	"	"	"	"	2.43	6.04	270	....	4
18	"	"	"	"	2.57	7.88	....	120	4
19	"	"	"	"	+	6.22	....	....	6
20	"	"	"	"	0	4.27	....	121	6
21	"	"	"	"	0	5.59	....	....	6
22	"	"	"	"	0	5.16	....	125	6
23	"	"	"	"	0	4.92	217	....	12
24	"	"	"	"	0	5.58	....	126	12
25	"	"	"	"	0	7.78	187	....	12
26	"	"	"	"	0	7.78	153	126	12
27	"	"	"	"	0	5.38	153	....	12
28	30	95	5	995	0	6.21	....	128	12
29	"	"	"	"	0	5.38	....	....	12
30	"	"	"	"	0	4.28	....	128	12
Dec.									
1	"	"	"	"	0	4.03	142	....	12
2	"	"	"	"	0	....	....	130	12
3	80	95	5	1195	0	5.82	142	....	12
4	"	"	"	"	0	3.69	....	130	12
5	"	"	"	"	0	4.38	....	....	12
6	"	"	"	"	0	5.00	211	135	12
7	"	"	"	"	0	6.63	....	....	12
8	"	"	"	"	0	5.88	168	130	16
9	"	"	"	"	0	4.79	....	....	16
10	"	"	"	"	0	5.83	....	130	0
11	"	"	"	"	0	3.80	....	....	16
12	"	"	"	"	0	....	187	130	16
13	"	"	"	"	0	7.52	....	....	16
14	"	"	"	"	0	8.11	....	128	16
15	"	"	"	"	0	10.80	146	....	16
16	"	"	"	"	0	9.66	....	125	16
17	125	95	5	1375	0	13.22	....	....	16
18	"	"	"	"	0	5.34	153	126	16
19	"	"	"	"	0	13.60	....	....	16
20	250	95	5	1875	0	15.59	168	128	16
21	"	"	"	"	0	34.44	....	....	8
22	"	"	"	"	0	21.58	....	128	16
23	"	"	"	"	0	30.84	306	....	16
24	90	137	25	1700	0	....	....	128	16
25	"	"	"	"	—	....	....	....	16
26	"	"	"	"	—	....	....	....	16
27	"	"	"	"	—	....	....	....	16
28	"	"	"	"	0	19.55	294	128	16
29	"	"	"	"	0	6.87	....	....	16
30	"	"	"	"	0	....	....	125	16
31	"	"	"	"	....	....	....	....	16

TABLE 7.—Continued

Date	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
1923									
Jan.									
1	90	137	25	1700	—	....	....	....	16
2	"	"	"	"	....	....	....	....	16
3	"	"	"	"	++++	....	385	120	16
4	"	"	"	"	++++	....	....	....	16
5	"	"	"	"	0	....	....	124	16
6	"	"	"	"	5.43	....	....	....	16
7	"	"	"	"	—	....	366	128	18
8	"	"	"	"	+	....	....	....	18
9	"	"	"	"	+	....	....	124	18
10	"	"	"	"	+	....	283	....	18
11	"	"	"	"	0	....	....	129	18
12	"	"	"	"	0	....	....	....	18
13	"	"	"	"	0	....	....	129	18
14	"	"	"	"	0	....	....	....	18
15	"	"	"	"	0	....	....	129	18
16	"	"	"	"	0	....	341	....	18
17	"	"	"	"	0	....	....	129	18
18	"	"	"	"	0	....	....	....	21
19	"	"	"	"	0	....	230	130	21
20	"	"	"	"	0	....	....	....	21
21	"	"	"	"	0	....	....	128	21
22	"	"	"	"	0	....	....	....	21
23	"	"	"	"	0	....	187	128	21
24	"	"	"	"	0	....	....	....	21
25	"	"	"	"	0	....	206	128	24
26	"	"	"	"	0	....	....	....	24
27	"	"	"	"	0	....	....	130	24
28	"	"	"	"	0	....	148	....	24
29	"	"	"	"	0	....	....	130	24
30	"	"	"	"	0	....	....	....	24
31	"	"	"	"	0	....	168	131	24
Feb.									
1	90	137	100	2000	0	....	159	....	24
2	"	"	"	"	0	....	....	132	24
3	"	"	"	"	0	....	....	....	24
4	"	"	"	"	0	....	214	131	24
5	"	"	"	"	0	....	182	....	24
6	"	"	"	"	0	....	153	133	24
7	90	137	200	2400	0	....	....	....	24
8	"	"	"	"	0	....	....	128	24
9	"	"	"	"	0	....	....	....	24
10	"	"	"	"	0	....	....	130	24
11	"	"	"	"	0	....	288	....	24
12	"	"	"	"	0	....	....	130	24
13	"	"	"	"	0	....	288	....	24
14	"	"	"	"	0	....	....	130	24
15	"	"	"	"	—	....	366	....	24
16	"	"	"	"	26.32	....	357	133	24
17	"	"	"	"	16.28	....	....	....	24
18	"	"	"	"	69.30	....	....	130	24
19	"	"	"	"	—	....	416	....	24
20	"	"	"	"	56.62	....	....	130	24
21	"	"	"	"	41.60	....	....	....	27
22	"	"	"	"	71.01	....	....	131	27

TABLE 7.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Feb.									
23	90	137	200	2400	33.06	....	....	....	27
24	"	"	"	"	46.20	....	....	133	27
25	"	"	"	"	21.60	....	....	....	36
26	"	"	"	"	28.98	....	....	131	36
27	"	"	"	"	32.76	....	349	....	36
28	"	"	"	"	63.55	....	....	131	40
Mar.									
1	"	"	"	"	29.88	....	....	....	40
2	"	"	"	"	30.37	....	357	135	40
3	"	"	"	"	16.32	....	....	....	40
4	90	159	100	2200	21.39	....	....	....	40
5	"	"	"	"	0	....	....	....	40
6	"	"	"	"	0	....	....	135	40
7	"	"	"	"	0	....	319	135	40
8	"	"	"	"	+	....	....	....	40
9	"	"	"	"	+	....	....	135	40
10	"	"	"	"	0	....	....	....	40
11	"	"	"	"	0	....	203	135	40
12	90	181	100	2394	0	....	....	....	40
13	"	"	"	"	0	....	....	135	40
14	90	253	90	3000	0	....	246	....	40
15	"	"	"	"	0	....	....	136	40
16	"	"	"	"	0	....	....	....	40
17	"	"	"	"	0	....	226	139	40
18	"	"	"	"	0	....	....	....	40
19	"	"	"	"	0	....	....	140	40
20	"	"	"	"	0	....	223	....	40
21	90	308	90	3500	0	....	....	135	40
22	"	"	"	"	0	....	258	....	40
23	"	"	"	"	0	....	242	136	40
24	"	"	"	"	0	....	....	....	40
25	"	"	"	"	0	....	178	138	40
26	"	"	"	"	0	....	178	....	40
27	"	"	"	"	0	....	....	140	40
28	"	"	"	"	0	....	199	....	40

patient's tolerance had risen, so that the unquestionable glycosuric influence of the added carbohydrate and protein was masked.

March 25, fat was increased so as to raise the total calories to 3000. This luxus diet evidently overtaxed the tolerance, for the plasma sugar rose to 0.2 per cent. within the short period of 3 days, when the experiment had to be broken off.

#### REMARKS ON TABLE 7.

This patient, after prolonged disregard of diet at home, was readmitted to the Institute on Oct. 31, 1922, with heavy glycosuria and plasma sugar of 0.555 per cent. He was treated until Nov. 8 without insulin. On a

diet of 30 gm. protein and 5 gm. carbohydrate, he excreted considerable sugar daily.

Insulin was first given in small doses of 1 to 2 units per day from Nov. 8 to 16. The combination of low diet and insulin reduced the glycosuria from about 20 gm. daily to about 4 gm. Increase of insulin to 4 units on Nov. 16 and to 6 units on Nov. 19 was sufficient to abolish glycosuria, and 12 units per day, beginning Nov. 23, reduced the plasma sugar to 0.142 per cent. on Dec. 1 and 3.

From Dec. 3 to 16, the diet was 80 gm. protein, 95 gm. fat, 5 gm. carbohydrate and 1195 total calories. The urine was continuously free from sugar and the plasma sugar was kept below 0.2 per cent. by an increase of insulin to 16 units per day. The patient felt fairly well, except for occasional weakness in the afternoons and evenings, and on two occasions he had symptoms of hypoglycemia.

Beginning Dec. 17, the effect of protein in considerable quantities was tried. 45 gm. with the extra calories was added at first, making a total of 125 gm., without appreciable rise in plasma sugar or increase in the patient's strength. A double allowance of protein, namely, 250 gm., was given from Dec. 20 to 24. The calories being thus considerably increased, the plasma sugar rose as high as 0.306 per cent. on the morning of Dec. 23, but in the afternoons there were still complaints of weakness, tremor and hunger. At 5 P. M. on Dec. 21, there were definite symptoms of hypoglycemia with sweating and tremor, and the evening dose of insulin had to be omitted. The influence of protein in raising the blood sugar or preventing hypoglycemic symptoms is thus seen to be not very strong.

On Dec. 24, the diet was changed to 90 gm. protein, 25 gm. carbohydrate and 1700 calories. With this reduction of protein and increase of carbohydrate and fat, the dosage of 16 units of insulin no longer sufficed to control hyperglycemia and glycosuria. With 18 units of insulin, the urine became sugar-free on Jan. 11, but hyperglycemia was not reduced until the dose was raised to 24 units on Jan. 25. It is probable that the gain in body weight to 130 lb. was one factor in the increased insulin requirement. It is evident nevertheless that a small increase of carbohydrate created greater tendency to hyperglycemia and glycosuria than large quantities of protein.

Larger quantities of carbohydrate were added to the diet, beginning Feb. 1. The first addition was of 75 gm. with the extra calories, making a total of 90 gm. protein, 100 gm. carbohydrate and 2000 calories. Glycosuria did not result, but instead there was a distinct fall in plasma sugar. A further increase of 100 gm. carbohydrate was given on Feb. 7. Insulin was kept unchanged at 24 units per day. For ten days glycosuria was absent, but the plasma sugar mounted to 0.366 per cent. on Feb. 15, and on the following day heavy glycosuria appeared abruptly. As much as 71 gm. of sugar was excreted on Feb. 22. Glycosuria was not stopped by increasing insulin to as much as 40 units daily from Feb. 28 to March 4. Hyperglycemia of over 0.3 per cent. was continuous. In this instance the effect of carbohydrate was slower than usual in becoming manifest, but powerful as usual.

On March 4, glycosuria was abolished by withdrawing 100 gm. carbo-



hydrate and reducing the total calories from 2400 to 2200. Hyperglycemia still persisted with the dosage of 40 units of insulin per day. Here the influence of a change of carbohydrate was both powerful and prompt.

TABLE 8  
Case No. 2229

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan.									
27	45	33	10	517	++++	.....	...	...	..
28	"	"	"	"	++++	.....	366	130	..
29	"	"	"	"	++++	.....	...	...	..
30	"	"	"	"	++++	.....	300	...	..
31	"	"	"	"	+++	.....	...	...	..
Feb.									
1	"	"	"	"	14.08	.....	...	...	2
2	"	"	"	"	0	.....	246	129	2
3	"	"	"	"	0	.....	...	...	2
4	"	"	"	"	0	.....	...	...	8
5	"	"	"	"	0	.....	126	128	8
6	"	"	"	"	0	.....	...	...	8
7	150	33	10	937	0	15.63	123	127	8
8	"	"	"	"	0	14.67	...	...	8
9	"	"	"	"	0	19.41	164	127	8
10	"	"	"	"	0	13.20	...	128	8
11	"	"	"	"	0	22.27	...	...	8
12	"	"	"	"	0	16.24	139	129	8
13	"	"	"	"	0	21.70	...	...	8
14	"	"	"	"	0	24.19	...	129	8
15	60	33	150	1137	0	.....	150	...	8
16	"	"	"	"	0	.....	...	128	8
17	80	151	80	2000	0	.....	...	...	15
18	"	"	"	"	0	8.93	...	134	15
19	"	"	"	"	0	.....	107	...	15
20	"	"	"	"	0	.....	129	131	15

#### REMARKS ON TABLE 8.

From admission on Jan. 27 to Feb. 1, this patient continued to show heavy (though decreasing) glycosuria on a diet of 45 gm. protein, 10 gm. carbohydrate and 517 calories. On Feb. 1, 2 units of insulin cleared the urine within 24 hours. With an increase to 8 units of insulin (4 units before breakfast and 4 units before supper) on Feb. 4, the plasma sugar was on the upper edge of normal (0.126 and 0.123 per cent.) in the morning samples taken before breakfast and before insulin. The evening analyses were in the neighborhood of 0.065 per cent. Hypoglycemic symptoms in the form of weakness, tremulousness and sweating occurred every evening. These were uncomfortable but not severe enough to demand emergency treatment.

Feb. 7, an increase of 105 gm. protein with the additional calories was



given, making the diet 150 gm. protein, 10 gm. carbohydrate and 937 calories. The protein was at first largely stored, and then catabolized in increasing quantities, as shown by the nitrogen analyses. Slight hyperglycemia appeared in the morning blood samples, but there was no glycosuria and the hypoglycemic symptoms continued each evening practically unchanged. Any specially powerful action of protein (specific dynamic or toxic) in causing sugar production is thus excluded.

On Feb. 15 and 16 the protein was reduced to 60 gm. and the carbohydrate increased to 150 gm. No glycosuria resulted, but the hypoglycemic symptoms ceased.

Feb. 17 to 20, the protein was raised to 80 gm., the carbohydrate reduced to 80 gm. and the fat increased greatly so as to make a total diet of 2000 calories. Increase of insulin to 15 units per day then caused no hypoglycemia. This greater tolerance for insulin can scarcely be explained by the glucose value of the diet, and is evidently due chiefly to the increase of total calories.

#### REMARKS ON TABLE 9.

With the low diets up to Dec. 1, glycosuria was stopped and the plasma sugar reduced to normal with 6 units of insulin per day, divided into doses of 2 units before each of the three meals.

Nov. 30 to Dec. 15, the effect of the addition of protein with its extra calories was tried. Dec. 3, the protein was increased from 40 to 80 gm., on Dec. 6 to 100 gm., on Dec. 8 to 120 gm., on Dec. 9 to 200 gm., and further to 300 gm. on Dec. 12. The fat was kept stationary at 25 gm. and carbohydrate at 10 gm. per day. During the early days of December, hypoglycemic symptoms began to appear each evening and gradually became more pronounced. The liberal protein increase from 120 gm. to 200 gm. per day did not serve to prevent them. On Dec. 8, while on a diet of 120 gm. protein, 10 gm. carbohydrate and 745 calories, the patient collapsed at 5 p. m. There was marked tremor of the hands, and he was very restless and talked irrelevantly. The plasma sugar was 0.046 per cent. He was given 20 gm. carbohydrate by mouth, and 25 gm. glucose intravenously at 5.10 p. m. Recovery was slow. At 8 p. m. he was still weak and excited, and for full relief required the feeding of 20 gm. more of carbohydrate. The following day, Dec. 9, the protein was increased to 200 gm. The insulin dosage was kept at 6 units in the belief that the protein was sufficient to prevent collapse, but such did not prove to be the case. During the morning the patient felt weak and listless. Plasma sugar analysis at 10.30 a. m. was 0.049 per cent. He collapsed at 5.30 p. m. while eating his supper. He was restless, irrational, unruly and noisy. He attempted to eat ravenously, but could not on account of tremor. He was immediately given 25 gm. of carbohydrate in the form of orange juice, and 20 gm. of levulose. He recovered very slowly, and it was not until 7 p. m. that he became normal. Plasma sugar taken at 8 p. m. was 0.055 per cent. The following day he was given the same diet of 200 gm. protein, 10 gm. carbohydrate and 25 gm. fat, and the insulin was omitted entirely. Glycosuria remained absent on this day and on the following day with the same diet and 4 units of insulin, but there was hyperglycemia of 0.175 per cent.

TABLE 9  
Case No. 1274

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Nov.									
16	25	5	10	185	++++	.....	...	109	1
17	"	"	"	"	++++	.....	405	...	2
18	"	"	"	"	++++	.....	...	107	2
19	"	"	"	"	++++	.....	416	...	4
20	"	"	"	"	+++	.....	...	102	4
21	"	"	"	"	++	.....	...	...	4
22	"	"	"	"	++	.....	366	105	4
23	"	"	"	"	0	.....	...	...	6
24	"	"	"	"	0	.....	...	105	6
25	"	"	"	"	0	.....	...	...	6
26	"	"	"	"	0	.....	234	105	6
27	"	"	"	"	0	.....	...	...	6
28	"	"	"	"	0	.....	...	107	6
29	"	"	"	"	0	.....	185	...	6
30	40	20	10	380	0	.....	...	109	6
Dec.									
1-2	"	"	"	"	0	.....	112	111	6
3	80	25	10	585	0	.....	125	...	6
4	"	"	"	"	0	.....	102	114	6
5	"	"	"	"	0	.....	...	...	6
6	100	25	10	665	0	.....	...	116	6
7	"	"	"	"	0	.....	.062	...	6
8	120	25	10	745	0	.....	*	115	6
9	200	25	10	1065	0	.....	.087†	...	0
10	"	"	"	"	0	23.42	112	109	4
11	"	"	"	"	0	19.05	175	...	6
12	300	25	10	1465	0	—	203	111	6
13	"	"	"	"	0	25.70	...	...	6
14	"	"	"	"	0	18.99	230	113	6
15	"	"	"	"	0	23.61	238	...	9
16	"	"	"	"	0	28.75	...	112	9
17	"	"	"	"	0	17.87	...	...	9
18	"	"	"	"	0	21.62	.092	114	9
19	"	"	"	"	0	21.93	175	...	6
20	"	"	"	"	0	20.63	...	116	6
21	"	"	"	"	0	31.17	...	...	4
22-23	90	111	25	1465	0	.....	209	112	9
27	"	"	"	"	.....	.....	178	...	9
28	"	"	"	"	0	6.77	178	111	9
29	"	"	"	"	0	5.94	209	...	9
30	"	"	"	"	0	8.93	...	108	9
31	"	"	"	"	0	—	...	...	9
1923									
Jan.									
1	"	"	"	"	0	13.17	...	106	9
2	"	"	"	"	0	12.54	168	...	9
3	"	"	"	"	0	15.88	...	104	9
4	40	111	75	1465	0	6.55	...	...	9

\*Collapsed at 5 p.m. Plasma sugar .046%. Given 65 gm. carbohydrate.

†Plasma sugar at 10.30 a.m., .049%; at 5 p.m., .043%. Given 40 gm. of carbohydrate; plasma sugar at 5.30 p.m., .055%.

TABLE 9. -Continued

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan.									
5	40	111	75	1465	+	10.29	349	105	9
6	"	"	"	"	+	4.1	...	...	9
7	"	"	"	"	0	6.87	288	108	9
8	"	"	"	"	+	5.66	...	...	9
9	"	"	"	"	+	5.23	...	109	9
10	"	"	"	"	+	—	294	...	9
11	"	"	"	"	+	—	319	110	9

On Dec. 12, the original insulin dosage of 2 units three times per day was given, and the protein increased to 300 gm. The patient felt much stronger and showed no signs of collapse. On Dec. 15, the insulin dosage was increased to 3 units 3 times per day on account of the appearance of hyperglycemia of 0.238 per cent., but as this increase again produced symptoms of threatened collapse, the original dosage of 2 units three times per day was resumed. The value of protein for preventing hypoglycemia is thus seen to be surprisingly slight, and by no means in proportion to its theoretical glucose value.

On Dec. 22, a general rearrangement of diet was made, the protein being reduced to 90 gm., the fat increased to 111 gm., and the carbohydrate to 25 gm., while the total calories were kept constant at 1465. With this alteration, the patient's physical condition was improved, and the plasma sugar remained slightly above normal. The urinary nitrogen excretion fell with the reduced protein intake. It will be noticed that these results and the higher insulin tolerance were found with a diet which was lower in glucose value (but higher in total calories) than that with which hypoglycemia had occurred.

Jan. 4, the diet was again changed by substituting 50 gm. of carbohydrate for 50 gm. of protein, keeping the total calories unchanged. Glycosuria resulted on the following day, and persisted in traces on the same insulin dosage.

The experiment shows that when the total calories are kept constant, considerable exchanges of protein, carbohydrate and fat can be made with relatively little effect upon glycosuria or the insulin requirement. The power of protein to prevent hypoglycemia is relatively feeble.

#### REMARKS ON TABLE 10.

On admission, March 5, 1923, this patient was given an observation diet of 60 gm. protein, 25 gm. carbohydrate and 1200 calories. The blood plasma on admission showed sugar of 0.370 per cent., a faint nitroprusside reaction, and heavy lipemia. Heavy glycosuria continued. The 1200 calorie diet was in excess of the tolerance, and for the first four days without the use of insulin, practically no control of the condition was gained.

On March 9, 8 units of insulin cleared the urine entirely within 24 hours. A plasma analysis on March 10 showed hyperglycemia of 0.326 per cent., but the lipemia appeared much less marked than on admission.

On March 14, the diet was changed to 60 gm. protein, 62 gm. fat, 100 gm. carbohydrate and 1200 calories. The insulin was kept at 8 units per

TABLE 10  
Case No. 2071

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Mar.								
5	60	95	25	1200	++++	370(a)	...	...
6	"	"	"	"	+++	...	120	...
7	"	"	"	"	16.59	366(a)	...	...
8	"	"	"	"	21.42	...	120	...
9	"	"	"	"	+	...	...	8
10	"	"	"	"	0	326(a)	118	8
11	"	"	"	"	0	...	...	8
12	"	"	"	"	0	...	120	8
13	"	"	"	"	0	214(b)	...	8
14	60	62	100	1200	0	...	120	8
15	"	"	"	"	0	...	...	8
16	"	"	"	"	+	...	120	8
17	"	"	"	"	+	298(b)	...	8
18	"	"	"	"	0	...	120	8
19	"	"	"	"	0	...	...	8
20	160	43	42	1200	0	...	123	8
21	"	"	"	"	0	250(b)*	...	8
22	"	"	"	"	0	...	122	8
23	"	"	"	"	0	178(c)†	...	8
24	"	"	"	"	0	...	125	8

(a)Lipemia 4 plus.

(b)Lipemia 2 plus.

(c)Lipemia 1 plus.

\*Plasma sugar at 7 p.m., .113%.

†Plasma sugar at 7 p.m., .097%.

day. This addition of carbohydrate without change in total calories caused traces of glycosuria on March 16 and 17, but with these exceptions the urine was free from sugar. Hyperglycemia of 0.298 per cent. was present on March 17, and moderate lipemia was still in evidence, in spite of the liberal carbohydrate and the use of insulin.

From March 20 to 24, the diet was again altered. 100 gm. protein was added, while 58 gm. carbohydrate was withdrawn. The total calories in the diet were kept at 1200, which called for a reduction in the fat from 62 gm. to 43 gm. The insulin dosage was kept unchanged at 8 units daily. The alteration in diet produced immediate effect upon the blood sugar. Hyperglycemia of 0.250 per cent. on March 21 showed decrease to 0.178 per cent. on March 23, and lipemia, which was 2 plus on the 21st, was

practically absent on the 23rd. At 7 P. M. on March 21 and 23, the patient had symptoms of hypoglycemia, namely tremor, sweating and nervousness. The plasma sugar readings during the attacks were 0.113 per cent. and 0.097 per cent. respectively, though it is not certain that these represented the lowest figures reached.

This experiment illustrates the familiar rule that carbohydrate creates the strongest tendency to hyperglycemia and glycosuria, while the influence of the theoretical equivalent in the form of protein (viz. 100 gm. protein for 58 gm. carbohydrate) is distinctly less.

TABLE 11  
Case No. 1341

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb. 28	40	44	10	600	....	...	...	..
Mar. 1	"	"	"	"	+	125	...	...
2	"	"	"	"	0	...	...	...
3	"	"	"	"	0	111	...	...
4	"	"	"	"	0	...	...	...
5	"	"	"	"	0	127	...	...
6	50	88	25	1100	+	...	34	...
7	"	"	"	"	++++	...	...	...
8	"	"	"	"	+++++	...	35	...
9	"	"	"	"	++	212	...	...
10	"	"	"	"	+++	...	35	...
11	50	7	100	663	22.72	...	...	...
12	"	"	"	"	++	...	35	4*
13	"	"	"	"	39.72	536†	...	4
14	"	"	"	"	20.85	...	34	4
15	"	"	"	"	31.20	...	...	4
16	"	"	"	"	39.72	178	35	4
17	"	"	"	"	24.95	...	...	4
18	"	"	"	"	50.40	...	34	4
19	"	"	"	"	24.00	223	...	4
20	150	7	42	840	12.27	...	34	4
21	"	"	"	"	+	...	...	4
22	"	"	"	"	.96	100	33	4
23	"	"	"	"	0	...	...	4
24	"	"	"	"	0	119	33	4

\*Insulin given b.d.

†1½ hours after a breakfast containing 50 gm. of glucose.

#### REMARKS ON TABLE 11.

From admission on Feb. 28 to March 5, this girl was kept free from glycosuria on a low diet of 40 gm. protein, 10 gm. carbohydrate and 600 calories, without insulin. Plasma sugar values were within normal range. March 6 to March 11, a general increase of diet to 50 gm. protein, 25 gm. carbohydrate and 1100 calories produced glycosuria.

TABLE 12  
Case No. 1328

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.								
15	30	15	5	275	.....	.....	.....	.....
16	"	"	"	"	56.00	429	116	.....
17	"	"	"	"	50.10	.....	.....	.....
18	"	"	"	"	54.40	.....	115	.....
19	"	"	"	"	.....	405	.....	.....
20	"	"	"	"	49.70	.....	115	.....
21	"	"	"	"	30.60	.....	.....	.....
22	"	"	"	"	29.12	.....	115	.....
23	"	"	"	"	19.00	334	.....	.....
24	"	"	"	"	24.92	.....	120	.....
25	"	"	"	"	10.80	.....	.....	4
26	"	"	"	"	+	294	120	4
27	"	"	"	"	+	.....	.....	4
28	50	71	40	1000	0	.....	127	4
Mar.								
1	30	15	100	655	25.80	341	.....	4
2	"	"	"	"	67.76	.....	130	4
3	"	"	"	"	23.20	.....	.....	4
4	"	"	"	"	43.26	.....	129	4
5	"	"	"	"	28.05	249	.....	4
6	"	"	"	"	23.23	.....	125	4
7	"	"	"	"	20.45	.....	.....	4
8	"	"	"	"	22.76	357	125	4
9	"	"	"	"	24.00	.....	.....	4
10	"	"	"	"	13.42	.....	120	4
11	"	"	"	"	12.32	334	.....	6
12	"	"	"	"	19.60	.....	125	6
13	"	"	"	"	23.20	.....	.....	6
14	30	80	5	900	0	223	128	6
15	"	"	"	"	0	.....	.....	10
16	"	"	"	"	0	.....	127	10
17	"	"	"	"	0	238	.....	10
18	"	"	"	"	0	.....	127	10
19	"	"	"	"	0	.....	.....	10
20	"	"	"	"	0	.....	129	10
21	"	"	"	"	0	214	.....	10
22	"	"	"	"	0	.....	132	10
23	"	"	"	"	0	199	.....	10
24	"	"	"	"	0	200	129	10
25	30	151	5	1500	0	162	.....	10
26	"	"	"	"	0	.....	128	10
27	"	"	"	"	0	.....	.....	10
28	"	"	"	"	0	.....	125	10
29	"	"	"	"	0	263	.....	10
30	"	"	"	"	0	.....	128	10
31	"	"	"	"	0	234	.....	10
April								
1	30	100	56	1244	0	.....	133	10
2	"	"	"	"	0	206	.....	10
3	"	"	"	"	+	.....	129	10
4	"	"	"	"	0	.....	.....	10
5	"	"	"	"	+	.....	131	10
6	"	"	"	"	+	.....	.....	10



TABLE 12.—*Continued*

Date 1923	DIET				Glyco- Suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
April								
7	30	100	56	1244	0	230	130	10
8	"	"	"	"	0	"	"	10
9	"	"	"	"	0	"	129	10
10	"	"	"	"	0	"	"	10
11	"	"	"	"	0	"	129	10
12	"	"	"	"	0	200	"	10
13	"	"	"	"	0	"	129	10
14	"	"	"	"	0	125	"	10
15	"	"	"	"	+	226	129	10
16	"	"	"	"	+	"	"	10
17	"	"	"	"	0	"	129	10
18	"	"	"	"	0	214	"	10
19	"	"	"	"	0	200	129	10
20	"	"	"	"	0	272	"	10
21	"	"	"	"	0	"	130	10
22	"	"	"	"	0	"	"	10

Beginning March 11, the protein was kept constant, the fat was reduced to 7 gm., and the carbohydrate was increased to 100 gm. per day. A sharp rise of glycosuria resulted immediately, and was not stopped by 4 units of insulin per day beginning March 12. The daily glucose output ranged from 22 to 50 gm. Also, with the lower calories the patient was weaker than on the 1100 calory diet. The well known disadvantages of excessive carbohydrate in the diabetic diet were thus illustrated.

March 20 to 25, 100 gm. protein was substituted for its theoretical equivalent of 58 gm. carbohydrate, thus changing the diet to 150 gm. protein, 7 gm. fat, 42 gm. carbohydrate and 840 calories per day. Glycosuria ceased quickly, and the plasma sugar fell to normal.

The experiment shows the weaker glycosuric effect of protein when substituted for its theoretical equivalent of preformed carbohydrate.

#### REMARKS ON TABLE 12.

This patient was admitted Feb. 15, 1923, with plasma sugar of 0.429 per cent. He was placed on a diet of 30 gm. protein and 5 gm. carbohydrate, and excreted more than 50 gm. of glucose on each of the first three days. The excretion was still 25 gm. on Feb. 24. Insulin was begun in dosage of 4 units on Feb. 25, and glycosuria was absent after Feb. 27, though hyperglycemia persisted.

A diet of 1000 calories was given on Feb. 28, but the plan was changed, and on March 1 a diet composed chiefly of carbohydrate was tried, in the form of 30 gm. protein, 15 gm. fat, 100 gm. carbohydrate and 655 calories. During the next ten days he excreted in the neighborhood of 25 gm. of sugar per day. Beginning March 11, 6 units of insulin per day proved insufficient to stop the glycosuria.

TABLE 13  
Case No. 1305

Date	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
1923								
Jan.								
19	35	15	5	295	14.72	...	116	4*
20	"	"	"	"	+	283	...	4
21	"	"	"	"	2.94	...	119	4
22	"	"	"	"	0	242	...	4
23	"	"	"	"	0	...	116	4
24	"	"	"	"	0	223	...	4
25	"	"	"	"	0	...	120	4
26	"	"	"	"	0	...	...	4
27	"	"	"	"	0	173	120	4
28	"	"	"	"	0	...	...	4
29	135	20	0	720	0	...	120	4
30	"	"	"	"	0	159	...	4
31	"	"	"	"	0	...	119	4
Feb.								
1	"	"	"	"	0	180	...	4
2	"	"	"	"	0	164	119	4
3	"	"	"	"	0	...	...	4
4	"	"	"	"	0	...	119	4
5	"	"	"	"	0	152	...	4
6	"	"	"	"	0	...	119	4
7	135	40	10	940	0	146	...	4
8	"	"	"	"	0	...	118	4
9	200	40	10	1200	0	132	...	4
10	"	"	"	"	0	157	117	4
11	"	"	"	"	0	...	...	4
12	200	10	40	1050	0	...	117	4
13	"	"	"	"	0	156	...	4
14	"	"	"	"	0	...	118	4
15	200	40	80	1480	0	143	...	4
16-18	"	"	"	"	0	...	117	4
19	"	"	"	"	0	168	...	4
20	"	"	"	"	0	...	117	4
21	"	"	"	"	0	173† 168 234†	...	0
22	"	"	"	"	0	166	116	0
23-25	"	"	"	"	0	...	116	0
26	"	"	"	"	0	184	116	0
27-28	"	"	"	"	0	...	116	0
Mar.								
1	100	40	138	1312	0	169	...	0
2	"	"	"	"	0	...	117	0
3	"	"	"	"	0	152	...	0
4-5	"	"	"	"	0	...	116	0
6	"	"	"	"	0	169	116	0
7	"	"	"	"	0	...	...	0
8	"	"	"	"	0	166	116	0
9	"	"	"	"	0	...	...	0
10	"	"	"	"	0	...	116	0

\*Insulin given b.d.

†Plasma sugar at 8 p.m.



TABLE 13.—*Continued*

Date	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
1923								
Mar.								
11	100	40	138	1312	+	...	...	0
12	100	140	128	2172	+	...	116	0
13	"	"	"	"	2.52	119	...	0
14	"	"	"	"	0	260†	117	0
15	"	"	"	"	+	{ 187 254† }	...	...
16	"	"	"	"	5.12	...	117	0
17	"	"	"	"	0	...	...	0
18	"	"	"	"	17.98	...	119	0
19	"	"	"	"	14.25	...	...	0
20	"	"	"	"	12.63	...	119	0
21	"	"	"	"	18.28	260	...	0
22	"	"	"	"	30.40	...	119	0
23	"	"	"	"	12.95	238	...	0
24	"	"	"	"	37.75	...	119	6
25	"	"	"	"	0	...	...	6
26	"	"	"	"	0	226	119	6
27	"	"	"	"	0	...	...	6
28	"	"	"	"	0	211	...	10
29	"	"	"	"	0	...	120	10
30	"	"	"	"	0	...	...	10
31	"	"	"	"	0	184	...	10

†Plasma sugar at 8 p.m.

Beginning March 14, a Newburgh type of diet was tried in the form of 30 gm. protein, 5 gm. carbohydrate and 900 calories. Though the total calories were thus higher than before, glycosuria ceased immediately, illustrating the stronger glycosuric influence of carbohydrate as compared with fat. On the other hand, an increase of insulin to 10 units daily failed to reduce the plasma sugar to normal.

March 25, the fat was increased from 80 gm. to 150 gm., while protein and carbohydrate were kept constant at 30 gm. and 5 gm. respectively, thus raising the total calories to 1500. A definite increase of hyperglycemia resulted.

April 1, an exchange was made of 51 gm. of fat for 51 gm. of carbohydrate. The increase of carbohydrate seemed to be approximately balanced by the reduction of total calories in this change, for though traces of glycosuria appeared on certain days, on the whole there was no important or lasting alteration shown in the plasma sugar level or otherwise.

The entire experiment with this patient, though it illustrates the stronger glycosuric influence of carbohydrate as compared with other foods, also indicates that the glucose value of the diet is by no means the only governing factor.

## REMARKS ON TABLE 13.

The patient was admitted to the Institute on Jan. 7, 1923, and until Jan. 19 was treated without insulin. The case proved to be one of

marked severity, since he excreted from 14 to 50 gm. of glucose daily on a diet of only 35 gm. protein and 5 gm. carbohydrate, with hyperglycemia from 0.270 to 0.366 per cent. The body weight decreased from 118 lb. to 116 lb., but there was a tendency to edema.

Jan. 19 to 28, 4 units of insulin were given with the same diet of 35 gm. protein and 5 gm. carbohydrate. Glycosuria disappeared entirely by Jan. 22, and the plasma sugar dropped to 0.173 per cent. on Jan. 27.

Jan. 29 to March 1, the addition of large quantities of protein was tried. First, 100 gm. protein, mostly in the form of egg albumin and casein, was given from Jan. 29 to Feb. 7. No carbohydrate was given, and the fat was kept restricted to 20 gm. per day. Insulin was kept unchanged at 4 units per day. The addition of this quantity of protein with its extra calories did not cause glycosuria or any marked effect upon the blood sugar.

On Feb. 7 and 8, the addition of 10 gm. carbohydrate and 20 gm. fat, making the total diet 135 gm. protein, 40 gm. fat, 10 gm. carbohydrate and 940 calories, showed no immediate effect, and on Feb. 9 a further addition of 65 gm. protein, with the extra calories, again failed to produce glycosuria or hyperglycemia.

From Feb. 12 to 14, a gram-for-gram substitution was made, by withdrawing 30 gm. fat from the diet and replacing it with 30 gm. carbohydrate. No effect was noted in the laboratory findings. The addition of 40 gm. carbohydrate and 30 gm. fat from Feb. 15 to 20 resulted in only a slight rise of hyperglycemia. On Feb. 21 the 4 units of insulin was withdrawn. The same diet was still tolerated, showing that the preceding negative results with successive food additions were explained by a rise of the patient's tolerance. This source of error should be guarded against in the planning and interpretation of all experiments of this kind.

On March 1, without insulin, 100 gm. protein was withdrawn and replaced by its theoretical equivalent in the form of 58 gm. carbohydrate. This alteration changed the diet from 200 gm. protein, 40 gm. fat, 80 gm. carbohydrate and 1480 calories to 100 gm. protein, 40 gm. fat, 138 gm. carbohydrate and 1312 calories. Although the total calories were reduced by this change, the increase in carbohydrate produced a gradual increase of hyperglycemia, and a trace of sugar appeared in the urine on March 11.

On March 12, 10 gm. carbohydrate was withdrawn and 100 gm. fat added, as the supposed glucose equivalent. This substitution changed the diet to 100 gm. protein, 140 gm. fat, 128 gm. carbohydrate and 2172 total calories. The sugar fluctuated at first, then increased, until as much as 37 gm. was excreted on March 24. Insulin was then administered again in doses of 6 units daily. This small amount of insulin was sufficient to abolish glycosuria within 24 hours. A further increase to 10 units on March 28 reduced the plasma sugar to 0.184 per cent.

The experiments on this patient show the greater glycosuric effect of preformed carbohydrate as compared with its theoretical equivalent in the form of protein, and also the marked glycosuric effect of an increase of fat and total calories.

#### REMARKS ON TABLE 14.

On admission to the Institute on March 4, 1923, the patient was given a diet of 50 gm. protein, 20 gm. carbohydrate, 102 gm. fat and 1200 calories.

She was kept on this diet for five days without insulin. She excreted 4 to 10 gm. of sugar daily, and her plasma sugars ranged from 0.234 to 0.250 per cent. The body weight was 76 lb. From March 9 to 14, the diet was kept the same, but 4 units of insulin was given daily, in doses of 2 units twice daily. The small amount of extract cleared glycosuria immediately, and reduced the plasma sugar to 0.148 per cent.

The effect of the addition of 100 gm. of protein, while keeping the calories unchanged by reduction of fat, was studied from March 14 to 20. Insulin was kept constant at 4 units daily. The addition of protein produced immediate return of glycosuria; 2 gm. of glucose was excreted on March 14, and 11 gm. on March 17. The plasma sugar rose to 0.253 per cent.

TABLE 14  
Case No. 1344

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Mar. 4	50	102	20	1200	.....	.....	.....	.....	.....
5	"	"	"	"	10.80	.....	250	.....	.....
6	"	"	"	"	5.85	.....	.....	76	.....
7	"	"	"	"	4.80	.....	234	.....	.....
8	"	"	"	"	6.02	.....	.....	77	.....
9	"	"	"	"	0	.....	.....	.....	4*
10	"	"	"	"	0	.....	†	78	4
11	"	"	"	"	0	.....	.....	.....	4
12	"	"	"	"	.....	.....	.....	77	4
13	"	"	"	"	0	.....	173	.....	4
14	150	58	20	1200	2.88	.....	148	75	4
15	"	"	"	"	+	.....	.....	.....	4
16	"	"	"	"	+	.....	.....	78	4
17	"	"	"	"	11.65	22.80	272	.....	4
18	"	"	"	"	3.78	.....	.....	78	4
19	"	"	"	"	4.77	17.38	230	.....	4
20	50	76	78	1200	21.64	12.97	263	79	4
21	"	"	"	"	14.93	9.37	.....	.....	4
22	"	"	"	"	19.17	8.78	206	78	4
23	"	"	"	"	21.02	8.84	.....	.....	4
24	"	"	"	"	17.25	7.27	.....	78	4
25	50	102	20	1200	2.81	7.66	.....	.....	4
26	"	"	"	"	0	6.1	.....	78	4
27	"	"	"	"	0	10.3	209	.....	4

\*Insulin given twice daily.

†Plasma sugar at 7 p.m., .157%.

March 20, the diet was again changed, by deducting 100 gm. of protein and adding its theoretical equivalent of 58 gm. of carbohydrate. The total calories were kept unchanged. This alteration changed the diet from 150 gm. protein, 58 gm. fat, 20 gm. carbohydrate and 1200 calories to 50 gm.

TABLE 15  
Case No. 1276

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Dec.									
3	30	10	0	210	0	.....	.....	.....	1*
4	"	"	"	"	0	.....	.....	109	1
5	"	"	"	"	0	.....	.....	.....	1
6	"	"	"	"	0	.....	294	107	1 ½
7	"	"	"	"	0	.....	.....	.....	1
8	"	"	"	"	0	.....	.....	109	4
9	"	"	"	"	0	.....	.....	.....	4
10	"	"	"	"	0	.....	.....	107	0
11	"	"	"	"	0	.....	189	.....	2
12	"	"	"	"	0	.....	.....	107	4
13	50	30	0	470	0	.....	189	.....	4
14	"	"	"	"	0	.....	.....	105	4
15	"	"	"	"	0	.....	.....	.....	4
16	"	"	"	"	0	.....	199	105	4
17	"	"	"	"	0	.....	.....	.....	4
18	"	"	"	"	0	.....	.....	105	4
19	"	"	"	"	0	.....	.....	.....	4
20	"	"	"	"	0	.....	206	106	4
21	"	"	"	"	0	.....	.....	.....	4
22	"	"	"	"	0	.....	.....	105	4
23	"	"	"	"	0	.....	211	.....	4
24	"	"	"	"	0	.....	.....	105	4
25	"	"	"	"	0	.....	.....	.....	4
26	"	"	"	"	0	.....	.....	105	4
27	"	"	"	"	0	.....	184	.....	4
28	"	"	"	"	0	.....	.....	103	4
29	"	"	"	"	0	.....	.....	.....	4
30	"	"	"	"	0	.....	.....	103	4
31	8	28	50	484	0	.....	187	.....	4
1923									
Jan.									
1	"	"	"	"	0	.....	.....	103	4
2	"	"	"	"	+	.....	.....	.....	4
3	"	"	"	"	+	.....	189	104	4
4	"	"	"	"	0	.....	.....	.....	4
5	"	"	"	"	+	.....	.....	103	4
6	"	"	"	"	0	.....	178	.....	4
7	7	128	40	1340	0	.....	.....	102	4
8	"	"	"	"	0	.....	.....	.....	4
9	"	"	"	"	0	.....	.....	103	4
10	"	"	"	"	0	.....	153	.....	4
11	"	"	"	"	0	.....	.....	102	4
12	"	"	"	"	0	.....	.....	.....	4
13	"	"	"	"	0	.....	.....	102	4
14	"	"	"	"	0	.....	138	.....	4
15	"	"	"	"	0	.....	.....	103	4
16	"	"	"	"	0	.....	.....	.....	4
17	"	"	"	"	0	.....	143	102	4
18	50	108	40	1340	0	.....	.....	.....	4
19	"	"	"	"	0	.....	.....	102	4
20	"	"	"	"	0	.....	169	.....	4
21	"	"	"	"	0	.....	.....	102	4

\*Insulin given b.d.

TABLE 15.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan.									
22	50	108	40	1340	0	....	175	....	4
23	"	"	"	"	0	....	....	102	4
24	"	"	"	"	0	....	....	....	4
25	"	"	"	"	0	....	180	101	4
26	"	"	"	"	0	8.67	....	....	4
27	125	108	40	1640	0	5.67	178	103	4
28	"	"	"	"	0	12.59	....	....	4
29	"	"	"	"	0	5.19	....	102	4
30	"	"	"	"	0	....	214	....	4
31	"	"	"	"	0	....	....	103	4
Feb.									
1	"	"	"	"	0	15.43	234	....	4
2	"	"	"	"	0	4.46	....	104	4
3	"	"	"	"	0	14.02	....	....	4
4	"	"	"	"	0	14.89	....	105	4
5	"	"	"	"	0	24.09	223	....	4
6	"	"	"	"	0	14.81	....	104	4
7	175	108	40	1832	37.44	26.91	326	....	4
8	"	"	"	"	31.20	24.24	....	103	4
9	"	"	"	"	13.86	18.17	....	....	4
10	"	"	"	"	31.62	23.35	334	105	8
11	"	"	"	"	0	16.63	....	....	8
12	"	"	"	"	18.24	23.94	326	106	8
13	"	"	"	"	15.75	21.66	....	....	8
14	"	"	"	"	....	....	326	106	12†
15	"	"	"	"	....	....	....	....	12
16	"	"	"	"	35.56	....	385	106	12
17	"	"	"	"	28.30	....	....	....	12
18	"	"	"	"	32.82	....	....	105	15
19	"	"	"	"	+++	....	375	....	15

†Insulin given t.i.d.

protein, 76 gm. fat, 78 gm. carbohydrate and 1200 calories. No change was made in the insulin. The additional carbohydrate produced heavier glycosuria, ranging from 17 to 21 gm. per day. On March 25, the diet was changed again to the original one of 50 gm. protein, 102 gm. fat, 20 gm. carbohydrate and 1200 calories. Sugar disappeared within 24 hours, with the same dosage of 4 units of insulin daily.

This experiment seems to show a descending order of glycosuric influence of foods. When the total calories are kept constant, the greatest tendency to glycosuria is created by carbohydrate; the theoretical equivalent in the form of protein has distinctly less influence, while the influence of fat is least of all.

## REMARKS ON TABLE 15.

On 30 gm. protein, 10 gm. fat and no carbohydrate, this girl was free from glycosuria with 1 unit of insulin per day, but had continuous hyper-

glycemia. With an increase to 50 gm. protein, 4 units of insulin kept glycosuria absent, but did not suppress hyperglycemia.

Dec. 31 to Jan. 5, the protein was reduced to only 8 gm., and 50 gm. carbohydrate was added, with no particular change in the fat. Traces of sugar appeared in certain urine specimens on three days, but the morning plasma sugars were practically unchanged. In this instance carbohydrate showed only a slightly stronger glycosuric effect when substituted for protein on a nearly gram-for-gram basis.

Jan. 7 to 17, 100 gm. fat was added and 10 gm. carbohydrate withdrawn, so as to raise the total calories to 1340, while keeping the supposed glucose value of the diet unchanged. The plasma sugar actually fell, the influence of the 10 gm. of carbohydrate predominating over that of the added calories in a case of only moderate severity for a short period of time. It is important that brief experiments in such cases shall not be interpreted as disproof of the influence of fat.

Jan. 18 to 26, the protein was increased to 50 gm., while fat was reduced so as to keep the total calories unchanged. The plasma sugar rose slightly, but the influence of the extra protein was trivial.

On Jan. 27, the protein was increased to 125 gm. and on Feb. 7 to 175 gm., and as the fat and carbohydrate were kept constant the total calories were thus raised to 1640 and 1832. The first addition resulted in hyperglycemia and the second in glycosuria, which was not entirely stopped by an increase of insulin to 15 units daily. The glycosuric influence of the protein was thus very pronounced, but due consideration must be given to the increase of total calories which was also involved.

#### REMARKS ON TABLE 16.

From Feb. 9 to 15, while taking 4 units of insulin per day, this girl was partially free from glycosuria but had marked hyperglycemia. On Feb. 16, 50 gm. carbohydrate was added with its calories, which brought the total diet to 50 gm. protein, 50 gm. fat, 100 gm. carbohydrate and 1050 total calories. This addition produced immediate increase of glycosuria to 57 gm. on Feb. 16 and 51 gm. on Feb. 18. The insulin dosage was increased to 8 units on Feb. 21, to 12 units on Feb. 25, and 18 units on Feb. 28. For several days she continued to excrete 10 to 12 gm. of sugar per day. By March 4, 18 units rendered the urine sugar-free, but on March 6, 9.3 gm. was excreted. The insulin requirement for the addition of 50 gm. of carbohydrate in this case therefore appeared to be at least 14 units.

From March 7 to 15, the 50 gm. of carbohydrate which was added on Feb. 16 was withdrawn, but the total calories in the diet were kept at 1050 by increase of fat. With the same insulin dosage of 18 units per day, glycosuria ceased and the plasma sugar fell to 0.187 per cent. As usual, fat created less tendency to glycosuria or hyperglycemia than the caloric equivalent of carbohydrate.

On March 16, the 50 gm. of carbohydrate was again added to the diet, and the calories kept unchanged by decrease of fat. Glycosuria returned promptly, and increased to as much as 41 gm. on March 22. By increasing the insulin dosage to 24 units on March 25, glycosuria was halted within 48 hours. Here it may be estimated that the insulin requirement for the



TABLE 16  
Case No. 539

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Feb.									
9	50	50	50	850	0	.....	.....	36	4†
10	"	"	"	"	0	.....	341	36	4
11	"	"	"	"	0	.....	.....	35	4
12	"	"	"	"	4 25	.....	.....	36	4
13	"	"	"	"	3 62	.....	326	36	4
14	"	"	"	"	0	.....	.....	36	4
15	"	"	"	"	0	.....	.....	36	4
16	50	50	100	1050	57 35	.....	.....	36	4
17	"	"	"	"	55 39	.....	.....	37	4
18	"	"	"	"	51 60	.....	.....	37	4
19	"	"	"	"	+++++	.....	312	36	4
20	"	"	"	"	+++++	.....	.....	37	8
21	"	"	"	"	45 30	.....	366	37	8
22	"	"	"	"	48 98	.....	.....	38	8
23	"	"	"	"	34 50	.....	.....	38	12
24	"	"	"	"	29 16	.....	375	38	12
25	"	"	"	"	21 76	.....	.....	38	12
26	"	"	"	"	18 55	.....	.....	37	18*
27	"	"	"	"	17 00	.....	.....	.....	.....
28	"	"	"	"	11 70	.....	.....	.....	.....
Mar.									
1	"	"	"	"	12 24	.....	341	39	18
2	"	"	"	"	10 54	.....	.....	39	18
3	"	"	"	"	6 72	.....	.....	39	18
4	"	"	"	"	0	.....	.....	39	18
5	"	"	"	"	+	.....	.....	40	18
6	"	"	"	"	9 30	.....	230	40	18
7	50	72	50	1050	0	.....	263	40	18
8	"	"	"	"	0	.....	.....	40	18
9	"	"	"	"	0	.....	.....	40	18
10	"	"	"	"	0	.....	.....	42	18
11	"	"	"	"	0	.....	.....	42	18
12	"	"	"	"	0	.....	.....	41	18
13	"	"	"	"	0	20 20	250	40	18
14	"	"	"	"	0	27 00	.....	40	18
15	"	"	"	"	0	8 00	187	40	18
16	50	50	100	1050	0	7 40	.....	40	18
17	"	"	"	"	+	7 17	.....	38	18
18	"	"	"	"	2 72	6 68	.....	40	18
19	"	"	"	"	25 65	10 28	.....	40	18
20	"	"	"	"	22 10	6 98	.....	40	18
21	"	"	"	"	22 55	7 39	326	40	18
22	"	"	"	"	41 85	8 29	.....	40	18
23	"	"	"	"	17 47	7 56	341	40	18
24	"	"	"	"	12 87	7 22	.....	41	24
25	"	"	"	"	2 0	7 2	375	41	24
26	"	"	"	"	2 75	.....	.....	.....	.....
27	"	"	"	"	0	.....	341	.....	.....
28	"	"	"	"	0	.....	.....	.....	.....
29	"	"	"	"	0	.....	.....	.....	.....
30	"	"	"	"	0	.....	220	.....	.....
31	"	"	"	"	0	.....	.....	.....	.....

†Insulin given b.d.

\*Insulin given t.i.d.

TABLE 17  
Case No. 783

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.								
1	60	95	25	1200	0	...	...	4*
2	"	"	"	"	0	283	82	4
3	"	"	"	"	0	...	...	4
4	"	"	"	"	0	...	83	6
5	"	"	"	"	0	...	...	6
6	"	"	"	"	0	...	83	6
7	"	"	"	"	0	182	...	6
8	"	"	"	"	0	230	83	6
9	60	62	100	1200	9.20	263	...	6
10	"	"	"	"	32.96	...	85	6
11	"	"	"	"	9.24	...	...	6
12	"	"	"	"	12.87	...	...	6
13	"	"	"	"	20.97	272	...	6
14	"	"	"	"	36.48	...	86	6
15	"	"	"	"	—	...	...	6
16	"	"	"	"	...	366	...	6
17	"	"	"	"	26.00	...	85	6
18	"	"	"	"	30.30	357	85	6
19	"	"	"	"	33.75	...	...	6
20	"	"	"	"	31.50	{ 312 405† }	84	6
21	"	"	"	"	49.17	349	84	6
22	"	"	"	"	15.17	...	...	6
23	"	"	"	"	19.27	314	...	6
24	"	"	"	"	12.00	...	84	12
25	"	"	"	"	11.16	...	...	12
26	"	"	"	"	11.50	306	86	12
27	"	"	"	"	0	...	...	12
28	"	"	"	"	0	...	83	15
Mar.								
1	"	"	"	"	0	156†	...	15
2	"	"	"	"	—	268	84	15
3	"	"	"	"	—	...	...	15
4-5	"	"	"	"	0	...	81	15
6	"	"	"	"	0	195	83	15
7	"	"	"	"	0	230†	...	15
8	80	86	100	1550	0	...	84	15
9	"	"	"	"	0	...	...	15
10	"	"	"	"	0	...	84	15
11	"	"	"	"	0	203	...	15
12	80	120	160	1800	0	...	83	15
13	"	"	"	"	0	...	...	15
14	60	151	100	2000	0	...	83	15
15	"	"	"	"	0	...	...	15
16	"	"	"	"	0	...	85	15
17	"	"	"	"	0	175	...	15
18	"	"	"	"	0	...	84	15
19	"	"	"	"	0	203	...	15
20	"	"	"	"	0	...	83	15

\*Insulin given b.d.

†Plasma sugar at 7 p.m.



TABLE 17.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Mar.								
21	60	151	100	2000	0	187	..	15
22	"	"	"	"	0	..	84	15
23	"	"	"	"	0	192	..	15
24	"	"	"	"	0	..	86	15
25	"	"	"	"	0	..	..	18
26	"	"	"	"	0	214	85	18
27	"	"	"	"	0	203	..	18
28	"	"	"	"	0	..	85	18
29	"	"	"	"	0	189	..	20
30	"	"	"	"	0	166	87	20
31	"	"	"	"	0	..	..	20
April								
1	100	133	100	2000	0	..	89	20
2	"	"	"	"	0	..	..	20
3	"	"	"	"	0	..	88	20
4	"	"	"	"	0	195	..	20
5	"	"	"	"	0	..	86	20
6	"	"	"	"	0	..	..	20
7	"	"	"	"	0	192	90	20
8	"	"	"	"	0	..	..	20
9	"	"	"	"	0	206	88	20
10	"	"	"	"	0	206	..	20

added 50 gm. carbohydrate was greater by 6 units than for the caloric equivalent of fat, at least in an experiment of this brevity. In a sufficiently prolonged test, it is not certain that the discrepancy would be as great.

## REMARKS ON TABLE 17.

This patient had hyperglycemia but not glycosuria on a diet of 60 gm. protein, 25 gm. carbohydrate and 1200 calories, with 6 units of insulin per day.

Feb. 9, the carbohydrate was increased to 100 gm., while the fat was reduced to keep the total calories unchanged at 1200. Glycosuria resulted immediately, and ceased only on Feb. 27, the fourth day after the insulin had been increased to 12 units. A further increase to 15 units failed to reduce the plasma sugar to normal, again illustrating the strain upon the pancreatic function represented by hyperglycemia.

From March 8 to April 1, the diet was gradually increased to 100 gm. protein, 100 gm. carbohydrate and 2000 calories. The insulin was also increased to 20 units per day, in order to keep the plasma sugar from rising above its usual level of about 0.2 per cent. As the influence of carbohydrate is rapid, and as glycosuria remained absent with 100 gm. carbohydrate and 15 units of insulin daily up to March 24, it seems prob-

TABLE 18  
Case No. 806

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Dec.								
28	40	51	7	650	+	...	...	...
29	"	"	"	"	0	263	...	...
30	"	"	"	"	0	272	39	3
31	"	"	"	"	0	...	...	3
1923								
Jan.								
1	"	"	"	"	0	173	39	3
2	"	"	"	"	0	...	...	3
3	"	"	"	"	0	141	40	3
4	"	"	"	"	0	...	...	3
5	"	"	"	"	0	148	38	3
6	40	45	20	650	0	...	...	3
7	"	"	"	"	0	122	38	3
8-9	"	"	"	"	0	...	39	3
10	"	"	"	"	0	139	...	3
11	40	32	50	650	0	...	38	3
12	"	"	"	"	0	206	...	3
13-14	"	"	"	"	0	...	39	3
15	"	"	"	"	0	189	40	3
16-17	"	"	"	"	0	...	40	3
18	"	"	"	"	2 10	...	...	3
19	"	"	"	"	0	209	39	3
20	"	"	"	"	0	...	...	3
21	"	"	"	"	0	184	40	3
22	"	"	"	"	0	...	...	3
23	40	32	75	750	0	...	40	3
24	"	"	"	"	13 86	214	...	3
25	"	"	"	"	22 28	...	40	3
26	"	"	"	"	23 56	283	...	3
27	"	"	"	"	12 75	...	40	3
28	"	"	"	"	3 23	...	...	5
29	"	"	"	"	4 07	254	40	5
30	"	"	"	"	13 86	...	...	5
31	"	"	"	"	16 20	349	39	10
Feb.								
1	"	"	"	"	5 96	349	...	10
2	"	"	"	"	0	...	41	21
3	"	"	"	"	0	189*	...	21
4	"	"	"	"	0	...	41	21
5	"	"	"	"	0	156*	...	21
6	"	"	"	"	0	...	41	17
7	"	"	"	"	0	138*	...	15
8	"	"	"	"	0	...	41	8 <sup>1</sup> / <sub>2</sub>
9	"	"	"	"	0	168*	...	6
10	"	"	"	"	0	...	40	6
11	40	115	75	1500	0	195	...	6
12	"	"	"	"	5 04	...	41	6
13	"	"	"	"	+	...	...	6

\*Hypoglycemic reactions in the afternoons and evenings.

†Plasma sugar at 5 p.m., .074%.

TABLE 18.—*Continued*

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.								
14	40	115	75	1500	11 90	228		6
15	"	"	"	"	+++	...		6
16	"	"	"	"	13 60	...	12	6
17	"	"	"	"	21 60	306		6
18	"	"	"	"	16 64	...	12	10
19	"	"	"	"	16 83	...		10
20	"	"	"	"	14 94	349	42	10
21	"	"	"	"	4 35	...		15
22	"	"	"	"	0	250	12	15
23	"	"	"	"	2 88	...		15
24	"	"	"	"	+	...	43	15
25	"	"	"	"	+	...		15
26	"	"	"	"	11 73	...	43	15
27	"	"	"	"	+	...		15
28	70	104	70	1500	11 20	234	44	15
Mar.								
1	"	"	"	"	++	...		15

able that the higher requirement in April was due chiefly or entirely to the increase of total calories.

The chief purpose of the experiment is to show the stronger glycosuric effect of carbohydrate as compared with the caloric equivalent of fat in the first period mentioned. An increase of 75 gm. carbohydrate evidently required 6 units of insulin more than the caloric equivalent of fat which it replaced.

#### REMARKS ON TABLE 18.

This patient was a child who had been under treatment for two years, and, although the diabetes was of severe type, had been kept in fair condition by low diet. For three months previous to admission, her diet was 40 gm. protein, 7 gm. carbohydrate and 650 calories, on which she barely maintained body weight. During the ten days previous to readmission, glycosuria was practically constant.

She was readmitted Dec. 28 in good condition except for traces of glycosuria. She was given her usual diet of 40 gm. protein, 7 gm. carbohydrate and 650 calories, and the day following glycosuria was absent. Three units of insulin was then given daily, which served to prevent glycosuria and reduce the plasma sugar close to normal limits, in tests taken as usual before breakfast. Her clinical condition was unchanged; in fact, she felt weaker than before insulin treatment, especially in the late afternoons, when she complained of especial weakness and occasionally sweating, but at no time were these symptoms alarming.

On Jan. 6, the diet was changed by adding carbohydrate without changing the total caloric value. The addition of 13 gm. carbohydrate did not

produce hyperglycemia or change her general condition. She still felt weak each evening. On Jan. 11, 30 gm. more carbohydrate was added, making a total of 50 gm. daily. This addition of carbohydrate reduced the fat ration from 45 to 32 gm. daily, in order to keep the total calories at 650. Three units of insulin was given daily as before. There was glycosuria only on Jan. 18, but the general level of plasma sugar was distinctly raised and hypoglycemic symptoms disappeared. It will be noticed that in this instance the difference between nearly normal plasma sugar and hyperglycemia amounted to as much as 43 gm. of carbohydrate.

Beginning Jan. 23, 25 gm. carbohydrate with the extra calories was added, which gave a total diet of 40 gm. protein, 75 gm. carbohydrate and 750 calories. Glycosuria resulted immediately. On Jan. 28, increases of insulin sufficient to control glycosuria, caused by the added carbohydrate, were begun. The dosage was increased to 5 units and further to 10 units on Jan. 31. Ten units were not sufficient, since hyperglycemia persisted, and 5.9 to 16.2 gm. of glucose was excreted daily. On Feb. 2, 21 units was given, which promptly arrested glycosuria and reduced the plasma sugar. This dose was excessive, as shown by marked hypoglycemic reactions each afternoon, from about 3 P. M. to supper. The patient complained of tremor, weakness, sweating and hunger. The pulse was slow; she was pale and restless. Crying spells were frequent. On Feb. 6, the dosage was reduced to 17 units, and on Feb. 7 to 15 units. On Feb. 8, a total of  $8\frac{1}{2}$  units was given, the evening dose being omitted on account of symptoms of collapse. On Feb. 9, there was a further reduction to 6 units daily, and sugar failed to appear in the urine, but hyperglycemia of 0.195 per cent. developed by Feb. 11. According to this test, an addition of 68 gm. carbohydrate required 3 units of insulin for its assimilation. This reckoning may be too low, since part of the assimilation may have been provided for by a rise of the patient's own tolerance.

Beginning Feb. 11, the protein and carbohydrate were left unchanged and fat added to raise the total calories to 1500. Glycosuria appeared on the following day, and was only partially controlled by increase of insulin to 15 units per day. On this basis, an addition of 83 gm. fat required at least 9 units of added insulin for its assimilation.

#### REMARKS ON TABLE 19.

From Dec. 31 to Jan. 11, slight glycosuria, ranging from mere traces to as much as 5.5 gm. per day, was present on a diet of 60 gm. protein, 40 gm. carbohydrate and 1600 calories, with 8 units of insulin per day. Plasma sugar analyses showed continuous hyperglycemia. Beginning Jan. 11, the dosage was raised to 12 units a day, so that within four days the urine became continuously free of sugar, and the blood sugar was reduced as low as 0.195 per cent.

The addition of sufficient fat, on Jan. 21, to raise the total calories from 1600 to 2000 produced immediate glycosuria—an unusual result, since the action of fat is generally much slower. As much as 40 gm. of sugar was excreted on Jan. 27. On Jan. 29, 18 units of insulin did not suffice to abolish glycosuria, and on Feb. 4, 24 units proved sufficient only to reduce the excretion to traces. Beginning Feb. 8, 30 units were given daily, so

TABLE 19  
Case No. 878

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Dec. 31 1923	60	133	40	1600	+	...	...	8
Jan. 1	"	"	"	"	+	...	76	8
2	"	"	"	"	+	...	76	8
3	"	"	"	"	+	263	76	8
4	"	"	"	"	+	...	77	8
5	"	"	"	"	0	...	77	8
6	"	"	"	"	0	...	76	8
7	"	"	"	"	+	...	75	8
8	"	"	"	"	0	...	77	12
9	"	"	"	"	3.58	263	77	12
10	"	"	"	"	5.51	272	77	12
11	"	"	"	"	0	...	77	12
12	"	"	"	"	8.12	230	76	12
13	"	"	"	"	0	...	77	12
14	"	"	"	"	+	...	77	12
15	"	"	"	"	0	195	77	12
16	"	"	"	"	0	...	77	12
17	"	"	"	"	0	...	77	12
18	"	"	"	"	0	...	77	12
19	"	"	"	"	0	...	77	12
20	"	"	"	"	0	...	77	12
21	60	177	40	2000	10.56	220	75	12
22	"	"	"	"	29.82	...	76	12
23	"	"	"	"	29.60	268	76	12
24	"	"	"	"	22.00	...	76	12
25	"	"	"	"	21.0	306	76	12
26	"	"	"	"	31.68	...	76	12
27	"	"	"	"	40.32	306	71	18
28	"	"	"	"	38.40	...	71	18
29	"	"	"	"	21.0	294	77	18
30	"	"	"	"	44.0	...	77	18
31	"	"	"	"	39.6	...	77	18
Feb. 1	"	"	"	"	33.9	...	77	18
2	"	"	"	"	27.5	...	78	24
3	"	"	"	"	0	334	78	24
4	"	"	"	"	23.40	...	78	24
5	"	"	"	"	16.65	...	78	24
6	"	"	"	"	16.0	270	80	30
7	"	"	"	"	+	...	80	30
8-15	"	"	"	"	0	...	80	30
16	60	151	100	2000	+	...	80	30
17	"	"	"	"	2.25	...	80	30
18	"	"	"	"	0	217	80	30
19	"	"	"	"	0	...	81	30
20	"	"	"	"	0	110*	81	30
21	"	"	"	"	0	...	81	30
22	"	"	"	"	0	...	81	30
23	"	"	"	"	0	...	81	30
24	"	"	"	"	0	{ 195 <sup>1</sup> 288 <sup>2</sup> }	81	30

<sup>1</sup> 7 a.m.<sup>2</sup> 10 a.m.

\* 8 p.m.

TABLE 19.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.								
25	60	151	100	2000	0	...	...	30
26	"	"	"	"	0	...	81	30
27	"	"	"	"	0	200	...	30
28	"	"	"	"	0	133*	82	30
Mar.								
1	"	"	"	"	0	...	...	30
2	"	"	"	"	0	...	82	30
3	"	"	"	"	0	...	...	30
4	120	151	200	2640	18.40	...	82	30
5	"	"	"	"	2.47	...	...	30
6	"	"	"	"	12.42	226	83	30
7	"	"	"	"	5.20	...	...	30
8	"	"	"	"	24.45	270	83	30
9	"	"	"	"	19.52	...	...	30
10	"	"	"	"	12.61	...	84	30
11	"	"	"	"	29.70	272	...	30
12	"	"	"	"	17.28	...	86	30
13	"	"	"	"	0	...	...	30
14	"	"	"	"	7.25	...	86	36
15	"	"	"	"	30.40	...	...	36
16	"	"	"	"	11.68	...	87	36
17	"	"	"	"	7.85	300	...	36
18	"	"	"	"	15.00	...	88	36
19	"	"	"	"	+	254	...	36
20	"	"	"	"	10.54	...	87	36
21	"	"	"	"	11.78	...	...	36
22	"	"	"	"	13.60	242	89	36
23	"	"	"	"	10.57	...	...	36
24	"	"	"	"	6.14	...	90	36
25	"	"	"	"	6.0	...	...	40
26	"	"	"	"	4.8	{ 254 115* }	90	40
27	"	"	"	"	7.8	254	...	40
28	"	"	"	"	8.9	...	92	42
29	"	"	"	"	9.9	...	...	42
30	"	"	"	"	12.1	272	92	42
31	"	"	"	"	—	...	...	42
April								
1	"	"	"	"	10.4	...	92	45
2	"	"	"	"	0	234	...	45
3	"	"	"	"	1.7	...	94	45
4	"	"	"	"	5.0	...	...	45
5	"	"	"	"	0	234	92	45
9	"	"	"	"	0	...	...	45
10	"	"	"	"	8.0	...	...	45
11	"	"	"	"	0	...	96	45
12	"	"	"	"	3.1	230	...	45
13	"	"	"	"	3.2	...	97	45
14	"	"	"	"	11.2	097*	...	45
15	"	"	"	"	+	...	98	48
16	"	"	"	"	5.4	230	...	48
17	"	"	"	"	0	...	98	48
18	"	"	"	"	3.4	...	...	48
19	"	"	"	"	3.3	230	98	48

TABLE 19.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight Lb.	Insuli- Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
April								
20	120	151	200	2640	+	...	...	48
21	"	"	"	"	5.4	...	100	48
22	"	"	"	"	+	...	...	50
23	"	"	"	"	0	230	100	50
24	"	"	"	"	0	...	...	50
25	"	"	"	"	0	...	100	50
26	"	"	"	"	0	...	...	50
27	"	"	"	"	0	...	101	50
28	"	"	"	"	0	...	...	50
29	"	"	"	"	0	...	102	50
30	"	"	"	"	0	178	...	50

that by Feb. 18 glycosuria had entirely disappeared. The diet was kept at 2000 calories, with 30 units of insulin daily, until March 3. During this period hyperglycemia was present in tests taken before breakfast, but during the evenings the patient had hypoglycemic reactions, in the form of the usual weak feelings, tremor and sweating, and on several occasions she became hysterical. Carbohydrate promptly relieved in each instance.

Beginning March 4, the protein and carbohydrate in the diet were doubled. This changed the ration from 60 gm. protein, 151 gm. fat, 100 gm. carbohydrate and 2000 calories to 120 gm. protein, 151 gm. fat, 200 gm. carbohydrate and 2640 calories. Within six hours sugar appeared in the urine, and heavy glycosuria was present until the insulin dosage was increased to as much as 45 units on April 1. This dosage was continued until April 15, when it was increased to 48 units. April 22, a further increase was made to 50 units, and the day following glycosuria ceased entirely.

This experiment shows: (1) that an increase of fat by 44 gm. (from 133 to 177 gm.) raised the insulin requirement by 18 units (from 12 units to 30 units per day); (2) that an increase of 60 gm. protein and 100 gm. carbohydrate, with the extra calories, raised the insulin requirement by 20 units (from 30 units to 50 units per day). In this particular experiment, therefore, the increase of insulin requirement by fat seems fully on a par with the increase required for protein and carbohydrate.

#### REMARKS ON TABLE 20.

Jan. 25 to 31, on a diet of 30 gm. protein, 5 gm. carbohydrate and 275 calories, with 2 units of insulin per day, this woman's glycosuria ceased but hyperglycemia persisted.

Feb. 1 to 24, the carbohydrate was increased to 75 gm., leaving the protein and fat unchanged, thus increasing the total calories to 555. Glycosuria resulted, but was stopped by an increase of insulin to 6 units. Under



TABLE 20  
Case No. 2215

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Jan.								
22	30	15	5	275	25 20	429	97	0
23	"	"	"	"	24.96	...	..	0
24	"	"	"	"	17.54	366	..	0
25	"	"	"	"	3.50	...	99	2
26	"	"	"	"	1 80	357	..	2
27	"	"	"	"	+	...	98	2
28	"	"	"	"	0	...	..	2
29	"	"	"	"	+	...	97	2
30	"	"	"	"	0	288	..	2
31	30	18	5	"	0	...	95	2
Feb.								
1	30	15	75	555	0	268	..	2
2	"	"	"	"	6.48	...	..	2
3	"	"	"	"	3 17	...	94	2
4	"	"	"	"	6.16	...	..	2
5	"	"	"	"	6.72	375	90	2
6	"	"	"	"	18.90	...	..	2
7	"	"	"	"	+	366	89	4
8	"	"	"	"	2 10	...	..	4
9	"	"	"	"	0	...	92	6
10	"	"	"	"	0	217	89	6
11	"	"	"	"	0	...	..	6
12	"	"	"	"	0	{ 254† 220 230† }	89	6
13	"	"	"	"	0	{ ... 200 217† }	..	6
14	"	"	"	"	0	{ 214 312† 218 }	90	6
15	"	"	"	"	0	{ 300† ... 270† }	..	6
16	"	"	"	"	0	{ 238 ... 270 }	89	6
17	"	"	"	"	0	{ 238† 250 129† }	..	6
18	"	"	"	"	0	{ 234 211† }	87	6
19	"	"	"	"	0	...	..	6
20	"	"	"	"	0	...	86	6
21	"	"	"	"	0	...	..	6
22	"	"	"	"	0	...	87	6
23	"	"	"	"	0	...	..	6
24	"	"	"	"	0	...	88	6
25	100	6	25	555	0	...	..	6
26	"	"	"	"	0	150	90	6
27-28	"	"	"	"	0	...	89	6
Mar.								
1	"	"	"	"	0	135	..	6
2-4	100	6	100	855	0	...	89	6
5	"	"	"	"	0	211	..	6



TABLE 20.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. . Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Mar. 6	100	6	100	855	0	...	90	6
7	"	"	"	"	0	211	..	6
8	"	"	"	"	0	{ 203 } 214†	90	6
9	"	"	"	"	0	...	..	6
10	"	"	"	"	0	124†	90	6
11	"	"	"	"	0	214	..	6
12	"	"	"	"	0	...	90	6
13	"	"	"	"	0	187	..	6
14	80	206	80	2500	0	...	89	6
15	"	"	"	"	0	...	..	6
16	"	"	"	"	+	...	90	6
17	"	"	"	"	+	300	..	6
18	"	"	"	"	3.64	...	89	6
19	"	"	"	"	15.72	...	..	6
20	"	"	"	"	33.00	...	91	6
21	"	"	"	"	37.20	375	..	6
22	"	"	"	"	28.68	...	93	6
23	"	"	"	"	27.72	375	..	6
24	"	"	"	"	24.50	...	92	6
25	"	"	"	"	++++	385	..	12

†Plasma sugar taken at 7 p.m.

these conditions of undernutrition, therefore, the assimilation of the added 70 gm. of carbohydrate seemed to require only 4 extra units of insulin.

During the latter part of this period various distributions of the insulin dosage were tried. Feb. 11 to 14, it was given in two doses per day, 3 units before breakfast and 3 before supper. Feb. 14 to 21 it was given in one dose of 6 units before breakfast. From Feb. 21 to the end of the experiment (March 25) it was given in three doses, 2 units before each meal. The chief difference was that the single dose of 6 units was less effective, as the plasma sugar both morning and evening was higher from Feb. 14 to 21. With every arrangement of the doses, however, glycosuria was absent and hyperglycemia present on the diet mentioned with 6 units of insulin.

Feb. 25 to March 1, the carbohydrate was reduced to 25 gm. and the protein increased to 100 gm. This change, with a slight reduction of fat, resulted in a diet which was practically identical with the preceding one in both glucose and caloric value. The sole important difference was that the glucose was now chiefly in the form of protein instead of in preformed carbohydrate. Within this short period of 5 days, the long-standing hyperglycemia was reduced to a nearly normal figure of 0.135 per cent., illustrat-

ing the weaker influence of protein as compared with the theoretical equivalent of preformed carbohydrate.

March 2 to 13, 75 gm. carbohydrate was added with its extra calories, thus raising the diet to 100 gm. protein, 100 gm. carbohydrate and 855 calories. The plasma sugar rose markedly, but glycosuria remained absent. It seemed evident that the high assimilation of carbohydrate and protein with the relatively small insulin dosage in a severe diabetic case was due to the low caloric value of the diet, which still represented under-nutrition for a woman who was freely up and about. Approximately 10 pounds of weight had been lost since the beginning of the experiment.

To test this supposition, the diet was changed on March 14 to 80 gm. protein, 80 gm. carbohydrate and 2500 calories. Here both protein and carbohydrate were reduced; the theoretical glucose value of the diet was slightly lower than before, and a great increase of calories had been produced only by the addition of 200 gm. of fat. Glycosuria began within 2 days, increased to amounts above 20 gm. and sometimes above 30 gm. daily, and was not stopped by an increase of insulin to 12 units. This experiment gives one of the strongest examples of the influence of fat and total calories, and belongs properly with this subject which is discussed in paper No. 3.

#### REMARKS ON TABLE 21.

Additions of carbohydrate were made to the diet beginning Nov. 30. For ten days previous, the patient was free from glycosuria, but had slight hyperglycemia, on a diet of 40 gm. protein, 5 gm. carbohydrate and 600 calories, with 2 units of insulin per day. From Nov. 30 to Dec. 3, she was also free from glycosuria, but with slight hyperglycemia on 45 gm. protein, 10 gm. carbohydrate and 800 calories, with 2 units of insulin per day. From Dec. 3 to Dec. 16, she was given 20 gm. of additional carbohydrate with the extra calories. The 2 units of insulin were sufficient to prevent glycosuria, but the plasma sugars showed a tendency to increase, and rose to as much as 0.233 per cent. by Dec. 8. With the increase of insulin to 4 units per day on Dec. 12, hyperglycemia decreased to 0.195 per cent. on Dec. 14. An extra 10 gm. of carbohydrate, with the added calories, was given daily between Dec. 16 and Dec. 22 without increasing the insulin dosage. The 4 units of insulin per day were not sufficient to prevent increase in plasma sugars, and on Dec. 21 sugar was present in the urine.

Beginning Dec. 22, 10 gm. of carbohydrate was added, making a total of 50 gm. per day. Glycosuria increased rapidly to as much as 11.4 gm. on Dec. 28, 11.5 gm. on Dec. 29, and 22.6 gm. on Jan. 1. Hyperglycemia continued to increase, and on the morning of Jan. 2 was 0.326 per cent. An increase of insulin to cover the extra carbohydrate was started on Jan. 3, raising the dosage to 8 units. Glycosuria was partially controlled, and hyperglycemia reduced as low as 0.270 per cent. by Jan. 11. When the insulin was increased to 12 units, beginning Jan. 11, only faint traces of sugar appeared in the urine during the day, and on several occasions the specimens were entirely clear. Sixteen units of insulin were given from Jan. 14 to Jan. 18, and proved sufficient to keep glycosuria absent. Hyperglycemia still persisted, but increases of insulin to 20 units

TABLE 21  
Case No. 174

Date	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insu- lin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
1922									
Nov.									
23	40	46	5	600	.....	.....	211	67	2
24	"	"	"	"	0	.....	.....	66	2
25	"	"	"	"	0	.....	184	.....	2
26	"	"	"	"	0	.....	.....	66	2
27	"	"	"	"	0	.....	.....	.....	2
28	"	"	"	"	0	.....	152	64	1
29	"	"	"	"	0	.....	.....	.....	1
30	45	64	10	800	0	.....	.....	65	2
Dec.									
1	"	"	"	"	0	.....	157	.....	2
2	"	"	"	"	0	.....	.....	64	2
3	45	64	30	880	0	.....	.....	.....	2
4	"	"	"	"	0	.....	.....	67	2
5	"	"	"	"	0	.....	200	.....	2
6	"	"	"	"	0	.....	.....	66	1
7	"	"	"	"	0	.....	.....	.....	2
8	"	"	"	"	0	.....	223	66	2
9	"	"	"	"	0	.....	.....	.....	2
10	"	"	"	"	0	.....	.....	67	.....
11	"	"	"	"	0	.....	.....	.....	2
12	"	"	"	"	0	.....	.....	67	4
13	"	"	"	"	0	.....	.....	.....	4
14	"	"	"	"	0	.....	195	65	4
15	"	"	"	"	0	.....	.....	.....	4
16	45	64	40	920	0	.....	.....	66	4
17	"	"	"	"	0	.....	260	.....	4
18	"	"	"	"	0	.....	.....	63	4
19	"	"	"	"	0	.....	.....	.....	4
20	"	"	"	"	0	.....	200	64	1
21	"	"	"	"	+	.....	.....	.....	1
22	45	64	50	960	+	.....	.....	68	4
23	"	"	"	"	+	.....	250	.....	4
24	"	"	"	"	0	.....	.....	68	1
25	"	"	"	"	+	.....	.....	.....	4
26	"	"	"	"	+	.....	270	67	4
27	"	"	"	"	+	.....	.....	.....	4
28	"	"	"	"	11.44	.....	.....	68	4
29	"	"	"	"	11.56	.....	.....	.....	4
30	"	"	"	"	0	.....	.....	68	4
31	"	"	"	"	0	.....	.....	.....	4
1923									
Jan.									
1	"	"	"	"	22.66	.....	.....	68	4
2	"	"	"	"	16.48	.....	326	.....	4
3	"	"	"	"	0	.....	.....	66	8
4	"	"	"	"	12.27	.....	.....	.....	8
5	"	"	"	"	+	.....	300	66	8
6	"	"	"	"	5.61	.....	.....	.....	8
7	45	60	50	920	.....	.....	.....	67	8
8	"	"	"	"	.....	.....	.....	.....	8
9	"	"	"	"	4.79	.....	.....	67	8

TABLE 21.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per cc.	Weight, Lb.	Insu- lin Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan.									
10	45	60	50	920	4.52	.....	...	...	8
11	"	"	"	"	4.39	.....	270	69	12
12	"	"	"	"	+	.....	272	...	12
13	"	"	"	"	+	.....	...	69	12
14	"	"	"	"	0	.....	...	70	16
15	"	"	"	"	0	.....	...	70	16
16	"	"	"	"	0	.....	270	...	16
17	"	"	"	"	0	.....	...	71	16
18	"	"	"	"	0	.....	230	...	20
19	"	"	"	"	0	.....	...	69	20
20	"	"	"	"	0	.....	209	...	20
21	"	"	"	"	0	.....	206	69	24
22	"	"	"	"	0	.....	171	...	24
23	"	"	"	"	0	.....	166	68	24
24	"	"	"	"	0	.....	171	...	24
25	"	"	"	"	0	.....	...	68	24
26	"	"	"	"	0	.....	187	...	24
27	"	"	"	"	0	.....	...	68	24
28	45	37	100	920	0	.....	...	...	24
29	"	"	"	"	0	.....	...	66	24
30	"	"	"	"	0	.....	171	...	24
31	"	"	"	"	0	.....	...	67	24
Feb.									
1	"	"	"	"	0	.....	...	...	24
2	"	"	"	"	0	.....	156	67	24
3	"	"	"	"	0	.....	...	...	24
4	"	"	"	"	0	.....	166	66	24
5	"	"	"	"	0	.....	...	...	24
6	"	"	"	"	0	.....	...	66	24
7	"	"	"	"	0	.....	168	...	12
8	"	"	"	"	0	.....	...	67	12
9	"	"	"	"	0	.....	...	...	12
10	"	"	"	"	0	.....	...	67	12
11	45	37	200	1320	0	.....	150	...	12
12	"	"	"	"	1.73	.....	152	67	12
13	"	"	"	"	0	.....	...	...	12
14	"	"	"	"	10.80	.....	160	69	12
15	"	"	"	"	...	.....	...	...	12
16	"	"	"	"	20.54	.....	...	69	12
17	"	"	"	"	22.32	.....	...	...	12
18	"	"	"	"	20.60	.....	...	69	12
19	"	"	"	"	0	.....	199	...	12
20	"	"	"	"	0	.....	...	69	12
21	"	"	"	"	9.02	.....	230	...	20
22	"	"	"	"	8.00	.....	...	69	20
23	"	"	"	"	9.69	.....	187	...	20
24	"	"	"	"	5.32	.....	...	69	20
25	"	"	"	"	6.67	.....	187	...	20
26	"	"	"	"	6.48	.....	101	69	20
27	"	"	"	"	0	.....	...	...	20
28	245	37	84	1649	0	.....	180	70	20
Mar.									
1-2	"	"	"	"	0	.....	...	68	20
3	"	"	"	"	0	.....	200	...	20

TABLE 21.—*Continued*

Date	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insu- lin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
1923									
Mar.									
4-5	245	37	84	1649	0	.....	...	68	20
6	"	"	"	"	9.45	.....	118	69	20
7-9	"	"	"	"	+	.....	...	79	20
10	"	"	"	"	6.40	.....	...	71	20
11	"	"	"	"	+	.....	254	...	20
12	"	"	"	"	6.23	.....	...	70	20
13	"	"	"	"	4.92	.....	270	...	20
14	"	"	"	"	+	.....	...	69	20
15	"	"	"	"	+	.....	223	...	20
16	"	"	"	"	+	.....	...	71	20
17	"	"	"	"	10.90	.....	...	...	20
18	"	"	"	"	+	.....	...	72	20
19	"	"	"	"	9.35	.....	250	...	20
20	"	"	"	"	5.88	.....	...	70	20
21	"	"	"	"	10.60	.....	288	...	20
22	"	"	"	"	+	.....	...	71	20
23	"	"	"	"	9.20	.....	234	...	20
24	"	"	"	"	17.25	.....	...	74	20
25	245	5	84	1360	12.3	24.8	288	...	20
26	"	"	"	"	1.2	24.7	258	72	20
27	"	"	"	"	8.9	30.3	...	...	20
28	"	"	"	"	7.1	19.6	...	74	20
29	"	"	"	"	7.2	11.8	300	...	20
30	"	"	"	"	5.7	35.1	...	75	20
31	"	"	"	"	0	18.7	...	...	20
April									
1	"	"	"	"	14.4	26.1	283	74	20
2	"	"	"	"	0	23.8	...	...	20
3	"	"	"	"	6.9	17.5	283	73	20
4	"	"	"	"	0	30.2	...	...	20
5	"	"	"	"	3.2	25.5	268	73	20
6	"	"	"	"	+	26.1	...	...	20
7	"	"	"	"	0	25.0	...	75	20
8	"	"	"	"	0	22.8	...	...	20
9	"	"	"	"	0	24.7	203	75	20

per day on Jan. 19, and to 24 units on Jan. 21 reduced the plasma sugar below 0.2 per cent. In this case, therefore, the addition of 45 gm. carbohydrate with the added calories increased the insulin requirement by approximately 22 units.

Beginning Jan. 28, 50 gm. of carbohydrate was added, but the total caloric value was kept at 920 by means of a reduction of the fat. This changed the diet from 45 gm. protein, 60 gm. fat, 50 gm. carbohydrate and 920 calories, to 45 gm. protein, 37 gm. fat, 100 gm. carbohydrate and 920 calories. The insulin was kept at 24 units per day. During this period sugar did not appear in the urine and only slight hyperglycemia, ranging from 0.156 to 0.171 per cent., was found in the analyses taken before breakfast. On Feb. 7, insulin dosage was actually decreased from 24 units

to 12 units, while the diet remained unchanged. Glycosuria still remained absent, and the plasma sugar analysis on the morning of Feb. 11 showed a reading of 0.150 per cent. The results of carbohydrate addition under these conditions are seen to be very different from those observed prior to Jan. 28.

An addition of 100 gm. of carbohydrate with the added calories was given beginning Feb. 11, with the same amount of insulin per day, namely, 12 units. Sugar appeared in the urine the following day to the extent of 1.7 gm., and increased to as much as 22 gm. by Feb. 17. The insulin dosage was increased to 20 units per day on Feb. 21. For the six following days sugar was excreted in the urine, but by Feb. 27, 20 units per day was sufficient to abolish glycosuria completely and to reduce the blood sugar to a normal value of 0.101 per cent. It is noteworthy that in this instance an increase of 100 gm. carbohydrate, even with the additional calories, seemed to increase the insulin requirement by only 8 units.

The withdrawal of 116 gm. of carbohydrate and the substitution of its theoretical equivalent of 200 gm. of protein was made on Feb. 28. This substitution changed the diet from 45 gm. protein, 37 gm. fat, 200 gm. carbohydrate and 1320 calories to 245 gm. protein, 37 gm. fat, 84 gm. carbohydrate and 1649 calories. Insulin dosage was kept unchanged at 20 units per day. The patient remained free from glycosuria until March 6, when 9.4 gm. of sugar was excreted. The same diet was given until March 24. Sugar was present in the urine each day, the quantities ranging from traces to 17.2 gm.

It was suspected that the apparently greater glycosuric influence of protein was due to the increase of total calories involved in the replacement of carbohydrate by its theoretical glucose equivalent of protein. This idea was tested by the nearly complete withdrawal of fat beginning March 25, so as to reduce the total calories to approximately the same amount as before the substitution of protein for carbohydrate was made. Glycosuria gradually diminished and was absent after April 7.

This experiment supports the following conclusions: (1) addition of carbohydrate increases glycosuria or the insulin requirement, probably more than the caloric equivalent in any other food; (2) this effect is not constant, but varies widely according to the composition of the total diet; (3) the effects of adding carbohydrate are different when the total calories are increased thereby and when the total calories are kept unchanged; (4) an increase of glycosuria or insulin requirement by protein is demonstrable only to the extent of its carbohydrate and caloric values; any additional effect due to specific dynamic, irritant or toxic influences as imagined by several writers is not demonstrable; (5) the difference between the insulin requirement for hyperglycemia and for normal blood sugar is demonstrable as usual.

### *Conclusions.*

1. Carbohydrate has a stronger glycosuric effect and creates a higher insulin requirement than the caloric equivalent of any other kind of food.



2. Protein ranks below preformed carbohydrate in respect to glycosuric effect and insulin requirement when the substitution is made on a basis of either equal caloric value or theoretical glucose content. Though hypoglycemia may be prevented by sufficiently large quantities of protein, this influence is surprisingly feeble and by no means in proportion to the theoretical glucose value.

3. Assumptions of a specially powerful glycosuric influence of protein, on the ground either of its specific dynamic or a supposed toxic action, are thus proved to be contrary to fact.

4. There is no constant scale of insulin dosage for the assimilation of any given quantity of carbohydrate. The ratio between grams of glucose and units of insulin varies widely not only in different patients but also in the same patient under different conditions.

5. Glycosuria and insulin requirement are governed to a very important degree by the total caloric value of the diet.





## CLINICAL OBSERVATIONS WITH INSULIN

### 3. The influence of fat and total calories on diabetes and the insulin requirement.

FREDERICK M. ALLEN, M. D.

*The Physiatrie Institute, Morristown, New Jersey.*

As dietary regulation is still necessary with insulin treatment, the problems of diet retain their full importance. Furthermore, insulin furnishes an important new means for their solution.

It was previously mentioned<sup>1</sup> that no two independent investigators in this country are treating diabetes in exactly the same way, and the differences in many instances are extreme. In general the undernutrition principle has maintained its dominant position, because it is supported theoretically by much experimental evidence, and because it has given unmistakable clinical benefits, and all its opponents are forced to make use of it in one form or another. On the other hand, the attacks upon it have never before been so strong, and this opposition continues to grow. Many upholders of undernutrition have become confused in their ideas, and mixtures of incompatible views constitute the worst feature of the situation. One pleasing condition, as mentioned, is that all the opponents are on terms of cordial personal friendship and are seeking the truth without partisan interest. Less fortunate are the circumstances, first, that these personal relations make debates embarrassing, and the desire to avoid any semblance of personal antagonism has resulted in a noticeable lack of direct criticism between the different parties; and second, that each group are so well satisfied with the validity of their own ideas that there is a dearth of attempts at decisive experimental demonstrations. A brief review of the present state of American literature will clarify these points and also permit an expression of the writer's attitude.

1. The fact—which has recently misled so many—that the materials metabolized are not necessarily identical with the materials of the diet and that metabolism continues even during fasting, is far from new. The foundation stone of the old classical treatment of diabetes was that fat metabolism is not greatly increased by fat feeding, and in a former review of the literature<sup>2</sup> the writer quoted von Noorden and others who proclaimed the harmlessness of liberal fat diets even in the presence of acidosis and impending coma for this theoretical reason. In this same

review, attention was called to the benefits of undernutrition observed empirically by a series of writers, and to the influence of fasting in reducing acidosis and raising carbohydrate tolerance, in defiance of the above theory. The writer has published many clinical and animal experiments in support of undernutrition and in opposition to the old theory. The old treatment fixed its view entirely upon sugar, and aimed to depress sugar metabolism by restricting sources of sugar, namely carbohydrate and protein, in the diet and substituting fat, and also to maintain body weight by feeding fat to prevent the burning of body fat. The new doctrine maintained essentially that the tolerance is affected by the total caloric value of the diet, and that undernutrition has specific benefits in diabetes which are not duplicated by the mere limitation of carbohydrate consumption or substitution of fat. This doctrine carries with it logically one of two conclusions; either that the internal secretion of the pancreas is concerned not merely with carbohydrate metabolism but also in an equally direct though less obvious way with the metabolism of all foods, or that sugar metabolism is in some way influenced by the metabolism of substances which supposedly form no sugar.

There is no doubt that practically identical food materials may be burned during fasting and on certain diets, and also no question has been raised of any difference of the manner of combustion according to the derivation from the food or from the body tissues. These metabolic laws were established a generation ago, but they have absolutely no bearing upon the point at issue. The burning of body substance constitutes undernutrition; the substitution of an identical food mixture prevents undernutrition. The writer regards it as a proved fact that undernutrition somehow—through the lowering of body weight, or of metabolism, or in any unknown way—exerts a specific beneficial influence upon the functional defect of diabetes. Diets which prevent undernutrition prevent its benefit. Whether the old or the new doctrine shall be found correct, it is desired here merely to emphasize the fact that the two are absolutely opposed and incompatible, that the writer set up the new one with full cognizance of the old one and of general metabolic laws, and that a decision between the two must rest not on theories but upon precise experimental evidence.

2. As proof of the fundamental newness of the writer's proposal, which was doubted by Billings and Woodyatt,<sup>3</sup> it is of interest that Woodyatt's latest important publication<sup>4</sup> shows a continued complete failure either to understand or to accept this view. In basing his thesis upon the unproved hypothesis that the internal pancreatic function is affected only by glucose, Woodyatt merely begs the question at issue. He has shown admirable skill in devising four sample diets of widely variable composition which are isoglucogenetic and nearly isocaloric, but the purpose of his work is to compose diets as high in calories as possible while limiting the glucose and at the same time preventing acidosis. In ignoring any specific benefit of undernutrition he is thoroughly consistent with his general position. Newburgh and his associates<sup>5, 6</sup> likewise aim frankly to prevent undernutrition by a liberal use of fat, and

their conception of sparing the internal pancreatic function is limited to the restriction of carbohydrate and protein. The existing friendly courtesy tends to obscure the actual scientific facts, as already mentioned, by complimentary personal references. Woodyatt<sup>4</sup> writes, "During the last few years the average of results obtained in the dietetic management of diabetes has been improved greatly through the work of Allen and Joslin, and the system they have developed is in some respects more logical and less empirical than any we have had heretofore." Newburgh and Marsh give similar credit. Other authors make generalizations to the effect that "the diet treatment of diabetes has been improved by Joslin, Allen, Newburgh and Marsh, Woodyatt and other workers." For scientific clarity, it is necessary to point out that these statements are incorrect. If the old doctrine of limitation only of glucose-forming foods is true, then the line of therapeutic advance runs directly from Naunyn on to Newburgh and Marsh and Woodyatt, and the undernutrition practiced by Joslin and the writer is a mistaken by-path. If the principle of total dietary restriction is correct, then Newburgh and Marsh, Woodyatt and their followers are opposing progress, because they have not accepted this principle and their diets are distinctly intended to prevent undernutrition. However friendly the workers, the beliefs are absolutely antagonistic and irreconcilable. One is right and the other is wrong; one must stand and the other must fall. The best scientific spirit seems to be to recognize this actual difference of opinion, and in a continued friendly fashion to seek for facts which will give a decision.

3. Turning to the evidence thus far submitted, the writer believes that Newburgh and associates started from incorrect premises in their supposed choice between high fat diets, high protein diets and indefinite continuance of undernutrition. Rations of 150 gm. or more of protein have never been used in any real undernutrition treatment, and the experiments showing that such excessive allowances cause glycosuria carry weight against Mosenthal<sup>7</sup> but not against Joslin or the present writer. Obviously, every patient must be brought into nutritive equilibrium if he is to live, and this result has been accomplished by Joslin and the writer in all diabetic cases except the few which are hopeless of control under any diet treatment. The advocates of undernutrition believe that the result is most successful, and especially most lasting, when undernutrition is used at first and when the total diet is kept permanently reduced in proportion to the severity of the diabetes. Joslin has used slightly more carbohydrate and has largely ignored hyperglycemia, while Newburgh and Marsh and the writer have agreed that it is best to keep the blood sugar normal if possible. With all of us, however, the ultimate maintenance diet, which is restricted in protein and carbohydrate, derives its energy chiefly from fat. For some reason—whether because of acidosis dangers, contrary to their original theories, or because of the distaste aroused in patients by excessive proportions of fat—Newburgh and Marsh have not actually given very high fat diets. Their records show examples of 1500 total calories or less for the final diets in severe cases, and give no support to the impression that these authors have been able

to keep such patients plump and strong and at the same time free from sugar and acetone. The degree of success obtained by Newburgh and Marsh seems to be readily explained by their initial undernutrition regime of 900 to 1000 calories, and by the restriction of total calories which followed automatically from their close restrictions of carbohydrate and protein, so that the final diets differed comparatively little from those of Joslin and the writer. With only casual mention of the belief that the extreme reduction of protein is inconvenient and unfounded, and that the slight excess of fat must prove injurious sooner or later, the chief objections to the work of Newburgh and Marsh may be stated as two. First, their doctrine that fat is harmless in the diabetic diet is fundamentally erroneous, and the publication of this doctrine has done harm by encouraging many physicians to feed far larger quantities of fat than Newburgh and Marsh themselves ever use. Second, wrong impressions have been created by the claim that certain diets consisting chiefly of fat act more powerfully than fasting in abolishing glycosuria, on the misleading theory that the protein catabolism of fasting is thus diminished. For example, Fulton<sup>8</sup> writes: "One very interesting and significant thing quite convincingly demonstrated by Newburgh was that patients who could not be made sugar-free by ordinary fasting would promptly become sugar-free when put upon this high fat diet." It was previously pointed out,<sup>9a</sup> and it seems desirable to reiterate, that Newburgh and associates have never reported a single instance of this kind under their direct observation. In two places,<sup>9</sup> claims of this kind have been made, based upon cases said to have failed to become sugar-free under fasting elsewhere and then successfully freed from sugar under Newburgh's diets. Nothing is commoner than to receive patients with such statements, based ordinarily on violations of diet, but the sugar clears up under accurate fasting or semi-fasting nevertheless. A comparison is apropos with the literature of D:N ratios and respiratory quotients. Lusk, Benedict and their followers have never obtained figures compatible with the formation of any important quantities of sugar from fat in diabetes; therefore, from this experience, they have held that such a process is non-existent, and that the different figures reported from some of the foremost European clinics have been erroneous and based upon violations of diet. The present writer has made many trials of fat feeding, both before and after the publications of Newburgh and Marsh, in the form of oil and butter days and all sorts of devices to protect the patient's strength and nutrition, and was forced to give them up not because of acidosis but because they interfered with gaining tolerance. Newburgh and Marsh have been fortunate to deal with cases mild enough to become symptom-free on their 900 calory diets. They make general assertions concerning youthful and severe cases, but examination of the detailed examples which they have given shows relatively mild ones—chiefly elderly patients or those who had lost little weight. Personal experience compels the writer to adopt a position regarding this matter like that of Lusk regarding D:N ratios; namely, to repeat that there is no such thing as a case of diabetes that can be cleared up by high fat diets and cannot be cleared



by fasting, but that on the contrary there are plenty of cases of such severity that they are cleared with extreme difficulty or not at all on Newburgh diets and are readily cleared by fasting or by rations limited to a few grams of protein and carbohydrate daily. If this statement shall be proved arbitrary and unjust, and if the claims of Newburgh and associates which seem to rest on hearsay shall be demonstrated as facts, the writer will cheerfully apologize. There is now, for example, an inviting opportunity for any investigator to prove that patients will take larger insulin dosage on fasting than on high fat diets.

4. The case against undernutrition has been most ably and clearly presented by Woodyatt.<sup>4</sup> The attack and the disagreement are fundamental and sweeping, and if his arguments are valid there can be no theoretical ground for undernutrition and no explanation for the clinical results actually obtained. Exceptions may first be taken to the general point of view.

(a) The definition of diabetes merely as an inability to utilize glucose cannot be accepted, because it would include, for example, phlorizin poisoning, with which Woodyatt has chiefly worked experimentally and to which he has consistently applied the name diabetes. Only one form of impaired glucose utilization is diabetes, and that is the form which results from deficiency of insulin. The prediction can be made with perfect positiveness that insulin will have no influence upon phlorizin glycosuria.

(b) The quantitative element in the definition, namely that the diabetic is able merely to use less glucose than the normal individual, also seems fallacious. The complete type of the disorder is "total" diabetes, in which the best authorities consider that no glucose whatever is utilized. Very few cases of human diabetes are "total"; in other words, the pancreatic islands are rarely destroyed or incapacitated completely, and accordingly some supply of insulin and some degree of carbohydrate assimilative power remain. Accordingly, the average human case represents a mixture between the abnormal and the normal state, and the mildness or severity of the case is determined by the extent to which the normal functional power is retained. A fundamental theoretical definition should not be based upon such a mixture.

(c) A small but important extension of this idea is represented in Woodyatt's practice of referring to his severely diabetic patient as "non-diabetic" during short periods in which the urine was free from sugar. There can be no clearness in this subject unless the distinction between diabetes and glycosuria is kept clear.

(d) There is no mystery about the carbohydrate "cures" of the past, which are explained partly by the "paradoxical law" mentioned below, but chiefly by the lowering of caloric intake ordinarily associated with them, especially by the undernutrition involved in a skim-milk or potato diet and in the "green days" preceding and following von Noorden's oatmeal days. These clumsy and out-of-date methods of applying temporary carbohydrate excess as an antidote for protein-fat excess never achieved

actual control of any cases except those which are now recognized in this country as mild, while the intelligent application of undernutrition has given long-lasting control of even the cases which were regarded as quickly and hopelessly fatal under the old methods.

(e) The statement that "the endocrine function of the pancreas, so far as we know it at all, is a single highly selective function having to do with the utilization of glucose and nothing else" ignores completely the writer's experiments extending over years, which have purported to show either that the pancreatic island function is concerned directly with the metabolism of other substances besides glucose, or that the utilization of glucose is influenced by the supply of other materials. Such a statement unsupported by any evidence merely begs the essential question that is under dispute.

Woodyatt has evidently been so confident of the impregnability of his theoretical views that he has felt able largely to ignore the necessity of formal proof. In looking for the foundation of such broad conclusions, we are surprised to find that it consists only of one case, which is frankly presented rather as an illustration than as a demonstration. This is the case of a man with severe diabetes, aged 26 years and weighing 45 kg., who was freed from glycosuria and acidosis by fasting and undernutrition, and at first had almost no food tolerance. After nine weeks of continuous undernutrition with occasional interspersed fast-days, the highest diet that could be taken without glycosuria during the tenth week seemed to be 67 gm. protein, 50 gm. carbohydrate, 56 gm. fat and 953 calories. Further changes were then tolerated as follows: first to 2.5 gm. protein, 24 gm. carbohydrate, 102 gm. fat and 1024 calories for 4 days; next to 11.5 gm. protein, 84 gm. carbohydrate, 162 gm. fat by a gradual increase through 10 days; next to 25 gm. protein, 84 gm. carbohydrate, 174 gm. fat and 2000 calories, which produced nitrogen equilibrium; finally a diet of 118 gm. protein, 28 gm. carbohydrate, 160 gm. fat and 2024 calories was tolerated for 8 days. This record and Woodyatt's interpretation seem to be open to criticisms as follows:

(a) The crux of the whole contention lies in the assumption of an excessive catabolism of body protein in the fasting of the emaciated patient, which could be spared by feeding fat, thus reducing the glucose formation upon which Woodyatt's attention is exclusively fixed. As no nitrogen analyses were performed, this crucial point is seen to be a pure supposition, and it is therefore well that the case is presented as an illustration rather than as proof. Woodyatt has speculated that as much as 161 gm. of body protein per day might have been catabolized. It is true that urinary nitrogen figures of this order have been found by Geyelin and Du Bois and others<sup>10</sup> in cases of extraordinary severity during short periods of intense acidosis, and also by Joslin<sup>11</sup> in cases of extremely severe and prolonged undernutrition shortly before death from inanition. Aside from a few such observations under special conditions, any reliable literature of "the azoturia of emaciated diabetics," to which Woodyatt appeals, seems to be non-existent. Neither the present writer nor anyone else, so far as revealed by the literature, has ever seen con-



tinued excessive nitrogen loss of this character in the short periods of fasting or undernutrition which clear up sugar and acetone in a case such as Woodyatt's, even granting that the patient may be extremely thin and weak. As far as known, the nitrogen figures always fall low with the disappearance of sugar and acetone and remain so at least for some months. Numerous examples are given by Joslin<sup>12</sup> and others. In the studies of Allen and Du Bois,<sup>13</sup> it may be noticed that Gerald S. weighed 40.15 kg. on the first day and 38.74 kg. on the last day of his 8-day fast. The urinary nitrogen during this time fell from 10.40 to 6.11 gm. John O'C., weighing only 32 kg., excreted only 6.02 gm. of urinary nitrogen on April 3, when his only food was 294 calories of alcohol. The nitrogen excretion of the emaciated sugar-free diabetics of the Rockefeller Institute series was always low. Patient No. 54 of this series,<sup>14</sup> a woman 29 years old and 5 feet 3 inches tall, weighed 65.5 kg. at the onset of her diabetes, and was reduced to 49 kg. by therapeutic undernutrition prior to admission. Owing to inability to acquire tolerance for any living diet, she gradually starved to death during nine months in the Rockefeller Institute Hospital, emaciating to a weight of 24 kg. Her daily nitrogen output averaged about 8 gm., representing a slight continuous negative balance on her usual carbohydrate-free diet of about 900 to 1000 calories, fully half of which was alcohol. Toward the close of February, 1915, a determined attempt was made to raise her tolerance by a diet of 300 to 400 calories, almost wholly of alcohol, for about 3 weeks continuously. The few nitrogen analyses obtained during this time were mostly 5 or 6 gm. daily. On the other hand, during the month before death the protein was increased slightly and the fat considerably, so as to make rations as high as 1500 calories. Marked glycosuria and acidosis promptly resulted, the nitrogen output rose as high as 12 gm., the negative nitrogen balance was not helped, and the clinical condition was made much worse. The reason why patients with hopelessly severe diabetes have been kept symptom-free on inadequate diets, even though death from inanition was inevitable, is because they could live both longer and more comfortably in this way and because addition of fat or any other food would bring back active symptoms and thus actually increase the loss of both nitrogen and body weight. In view of the number and uniformity of such findings, Woodyatt's assumption unsupported by any figures is scarcely permissible, and the burden of proof rests strongly upon anyone who assumes the existence of a consistently high nitrogen loss. Though Woodyatt unfortunately omits the height of his patient, the above mentioned patients were evidently as emaciated or more so, also they had more severe diabetes which required longer fasting to clear up. If protein catabolism in emaciated diabetics went on ordinarily at the rate alleged, the extremely severe cases described in paper No. 1 of this series could never have survived the extremely severe measures required for the control of their condition. The ideas of Woodyatt and also of Newburgh and Marsh concerning this catabolism appear to be unfounded and grossly exaggerated.

(b) Regardless of the degree of emaciation or the nitrogen excretion,

the writer's experience is that undernutrition still tends to raise the food tolerance, and increasing the total caloric intake with fat or any other food tends to lower it, even when allowance is made for the hypothetical glucose value of the fat. The writer agrees with Newburgh and Marsh that ratios between fat and carbohydrate can largely be ignored in these emaciated sugar-free patients, at least temporarily, and that large fat additions may produce no serious acidosis. The trouble arises not from dangerous acidosis but from the tendency to glycosuria created by the fat, though the latter according to current speculations should diminish the supply of glucose by reducing protein catabolism. These observations are so numerous and uniform that any opposing claims should be based upon reports of cases in sufficient number and detail to warrant scientific conclusions.

(c) The confusion of carbohydrate combustion and carbohydrate tolerance must be regarded as inaccurate and misleading. The fact is beyond question that the more glucose is introduced into the normal organism, the more will be utilized or retained, and the increment of excretion never becomes equal to the increment of dosage. The writer<sup>15</sup> long ago called attention to this as a paradoxical law of glucose. In proportion as the organism becomes diabetic (i. e., deficient in pancreatic island function) this power of response to increased functional demands is lost, but it is retained in some degree by all diabetic patients or animals whose disorder is not "total" or almost so. It accordingly existed in Woodyatt's patient, who excreted some sugar when given only 20 gm. or less of carbohydrate, and averaged an excretion of 85 gm. on a higher diet of which the glucose value was calculated at 142 gm., leaving at least 57 gm. daily apparently utilized. It cannot be admitted that either this figure or the 112 gm. subsequently calculated represented the maximum power of temporary utilization, or that such utilization was fixed and invariable. On the contrary, the feeding of 400 or 500 gm. of carbohydrate with a minimum of other food would have demonstrated a still higher glucose retention, and Woodyatt's very broad conclusions can be overturned by making suitably broad variations in the experimental conditions. The most important consideration, however, is that this overtaxing of the feeble residue of assimilative function is harmful in breaking down this function, and, as the injury from pancreatic overstrain is recognized also by Woodyatt, it is surprising that he failed to make a correct distinction between temporary forced combustion and actual tolerance.

(d) The contrast between theory and practice is striking. At the beginning of his paper, Woodyatt gives supposed reasons why fasting cannot possibly have a different effect than a diet furnishing the same materials for metabolism, and in the closing pages he calculates that fasting involved a loss of perhaps 161 gm. of body protein per day and a correspondingly excessive glucose formation. But in the management of his patient, whenever glycosuria appeared, one or two fast-days constituted the actual means employed to check it. Close study of this case reveals nothing but the perfectly familiar building up of tolerance by nine weeks of undernutrition. Woodyatt's assumption of an enormous

destruction of body protein places him in a difficult position, for the entire explanation which he offers for the phenomena falls if such a loss is found non-existent. He concedes (p. 139) that there may have been an actual rise of tolerance during the first part of the treatment. This is the part in which undernutrition was used, and he leaves open the question why the tolerance rose then and not later when the high diets were given.

(c) The benefits of undernutrition, as exemplified by Woodyatt's case and verified by a host of other physicians and patients, can be overthrown only by two clinical demonstrations. First, the high diets employed at the end of this case should be used at the beginning, and it should be shown that such diets clear up the symptoms as well as or better than undernutrition. After the condition is thus controlled, fasting or undernutrition should be imposed for a week or two, and it should be shown that huge waste of nitrogen develops and that the tendency to glycosuria is increased. Everybody familiar with undernutrition knows that the results are the opposite. Second, the high diets, as finally employed, should be continued for much longer than 8 days. Absence of glycosuria for 8 days is no proof that a diet is actually tolerated, particularly in the absence of blood sugar analyses. Especially the harmful influence of fat is usually slow in manifesting itself, as the writer has many times pointed out. The tolerance built up through nine or ten weeks would not necessarily be broken down by a slightly excessive diet for a few days. Woodyatt's case was not of extreme severity, as compared with the cases described in this Institute in which a considerable tolerance has finally been built up by undernutrition; but it was sufficiently severe that the writer seriously doubts that the patient in question ever tolerated the diet of over 2000 calories for any long time.

It is natural and excusable for any individual author to theorize along the lines of his favorite beliefs. It is only surprising that these theoretical dietary proposals, with no support except one inconclusive case, should have been accepted by such a large proportion of students of diabetes and metabolism in this country without a word of criticism, without a single attempt to verify their correctness, and with complete disregard of the numerous opposing facts.

5. An idea entertained by some is that the old and new doctrines can be reconciled by an assumption that a higher total metabolism usually involves a higher glucose metabolism, and that the benefits of undernutrition can be thus explained in comparison with the old-fashioned treatment, but that still further benefits are obtainable by the recent methods for a more cautious and skillful use of fat in depressing glucose metabolism. The work of Atkinson<sup>16</sup> is highly important as the first valid demonstration that higher fatty acids can to some extent under some conditions be transformed into sugar in the animal organism; but an assumption that a higher total metabolism involves a higher glucose metabolism on account of a conversion of fat into glucose is excluded by the well known evidence against any such conversion on a large scale. Wilder<sup>17</sup> has given support to the benefit of a reduced nutritional level

in diabetes, and has offered an explanation in terms of lowered metabolism alone. The writer, with Du Bois, was among the first to study the fall of metabolism accompanying the clearing of diabetic symptoms with undernutrition, and is sympathetic to this association. Metabolism is raised and diabetes aggravated not only by high diet but also by fever and by hyperthyroidism. Nevertheless, it is impossible to regard the metabolic level as the sole determining factor, for the following reasons. The raising of metabolism by exercise does not lower the diabetic tolerance but if anything raises it. High protein feeding, which increases metabolism, does not create a greater need for insulin than an equivalent amount of carbohydrate, and the unfounded fears of protein and extreme restrictions of protein in the diabetic diet are thus still further discredited. When an obese patient is reduced by a number of pounds of fat but at the same time has his flabby muscles developed by exercise, the basal metabolism is probably not diminished and perhaps is slightly increased, but the food tolerance is greatly raised nevertheless. When, at the other extreme, emaciated patients are subjected to long and rigorous undernutrition, the possibility must be faced of their reaching a condition in which their protein metabolism is increased, with an accompanying increase of glucose formation and of total metabolism. Nevertheless, in defiance of any theories, the fact must be strongly emphasized that these patients continue to gain tolerance with undernutrition even under these circumstances, and this gain of assimilative power is hindered by attempts to reduce the total or glucose metabolism by feeding fat. An illustration of the practical working of this theoretical doctrine is given by the experience of Campbell.<sup>18</sup> His patient W. M. failed to become free from glycosuria on a diet of 38 gm. protein, 39 gm. carbohydrate, 145 gm. fat and 1613 calories. His patient M. T. had otitis media, but it is noteworthy that on a "basal" diet of 24 gm. protein, 26 gm. carbohydrate, 90 gm. fat and 1010 calories she excreted 34 gm. of sugar, but was free from glycosuria on "non-nutrients." These diets follow exactly the plan of Woodyatt and Wilder in respect to the alleged ketogenic-antiketogenic balance, and they are so moderate in their content of individual and total foodstuffs that they probably did not raise the total metabolism to any important degree. The highly significant fact is not only that these "basal" diets failed to control the glycosuria with the well-known efficacy and promptness of fasting or semi-fasting, but also after a number of days in hospital both patients went into coma and were only saved by insulin. No instance of this kind has occurred in the entire history of the Physiatrie Institute, unless the diabetes was approximately "total" or some very serious infection was present, and Joslin's record is equally clear. The middle ear infection in the second case is a factor to be considered, but as the patient so readily became sugar-free on fasting it is improbable that she would have gone on to coma had she received the protein that was actually given, the same or a little more carbohydrate, and no fat whatever. The first case was uncomplicated. A number of physicians known to the writer have narrated experiences of this kind which have not been published. Such cases are an interesting confirma-



tion of the former evidence that diets which aim to prevent undernutrition do not give the therapeutic results of undernutrition, and also that moderate allowances of fat are not so harmless as supposed by Woodyatt, Wilder, Newburgh and others in respect to the production of both glycosuria and acidosis.

It appears that the opponents of undernutrition have placed too much reliance upon their interpretation of certain metabolic laws and upon clinical observations of an inconclusive type, and not enough upon exact proof of their views. The writer is not flattered by the inference that the undernutrition treatment was set up in ignorance of these rudimentary metabolic principles. The innovation consisted precisely in the demonstration that undernutrition accomplishes certain results that are not explainable by the theories which have dominated the older treatment of diabetes. The dispute pertains in no way to the established laws of metabolism, but only to the preconceived and unproved ideas of authors that the diabetic disturbance is limited to the glucose molecule and that all other food elements can be ignored. The theory of the undernutrition treatment is that the diabetic condition is affected by all energy carriers of the diet and by the body weight, either because the pancreatic island function is directly concerned in total metabolism, or because the supply of other food materials in some way influences the utilization of glucose. From the very outset the writer attempted to work toward a solution of the fundamental problems here involved, as shown by the several series of experiments still in course of publication,<sup>19</sup> but the obstacles placed in the way of the research prevented reaching a conclusion. The general doctrine, however, leaving open the choice between its two corollaries, is established not only by much clinical experience concerning obesity, undernutrition, etc., but also by animal experiments<sup>19, 20</sup> and by precise clinical proofs that diabetic symptoms can be produced by additions of fat to the diet,<sup>21</sup> even when allowance is made for the supposed glucose equivalent of such fat,<sup>22, 23</sup> and likewise by the addition of alcohol<sup>22, 24, 25</sup> which is presumed to be directly convertible into neither glucose nor acetone. These numerous experiments have never been refuted, but have merely been ignored by authors who found them incompatible with their theories.

Two new conditions are created by the splendid discovery of insulin.

First, though all the parties to this debate have believed in the pancreatic origin of diabetes and have planned their diets with

a view to reducing the endocrine labor of the pancreas, it must be recognized that "over-strain of the island function" has remained an embarrassingly vague expression to most minds, even after demonstration of the significance of hydropic degeneration. Furthermore, the diet experiments are long and inconvenient, and the animal experiments still more difficult, and authors have generally continued to adhere to their own methods and to make interpretations to suit their own theories. Now, with the actual internal secretion of the pancreas in our hands and quantitatively measurable within reasonable limits, the first thought that must suggest itself is that we have the means for bringing these matters to a show-down. It becomes feasible to determine quantitatively the demands for insulin created by different diets, and thus to deduce which method is best calculated to spare a weakened pancreatic function, in a definite manner which may be hoped to break the existing deadlock of opinions and provide a common basis of proof and agreement.

Second, we stand now at the threshold of the investigation of one of the most important hormones of the body, and the one which is more intimately concerned in cellular nutrition than any other glandular product that is known. Enormous labor may be wasted and possible advances long postponed if this investigation starts on wrong lines. The reason for the frankest possible discussion and criticism at this time is to be found not in the love of polemic and personal justification which is an unfortunate feature of science, but in the importance of establishing right premises at this particular stage. Already it has been reported from Hopkins' laboratory<sup>26</sup> that insulin accomplishes a conversion of the alpha-beta to the gamma form of glucose, and other activities of chemists along such lines may be anticipated. Whatever success may attend efforts to explain the impaired utilization of glucose, which is the most obvious characteristic of diabetes, the facts which have been mentioned seem difficult to explain by this one impairment, and no hypothesis concerning insulin should be set up which cannot cover all the facts.

The present experiments pertain primarily to the effects of fat feeding upon diabetic patients. Concerning technique, it may suffice to say that the sugar in blood and urine was determined by Benedict's methods, and the entire plan was the same as in the experiments previously reported, except for the measurement of results in terms of insulin. Variations in potency of the insulin

were excluded as far as possible by using the same lot throughout an experiment or by changing only under controlled conditions, so that it is believed there is no danger of the results being explained by irregularities from this source. The chief difficulty or mistake which investigators may encounter in undertaking to repeat these observations will consist merely in the selection of cases and the planning of the experiments. The effects of fat are slower than those of carbohydrate or even of protein. They are most prompt and obvious in the severest cases, which therefore are generally most convenient for study. In relatively mild cases, such as those described by Newburgh and Marsh, they may be missed for a time but make their inevitable appearance if a sufficient number of months be allowed. Case No. 25 (Table 17) in this series is an example of the slowness of results possible even in cases of severe type. The greater part of the so-called "spontaneous" progressiveness of diabetes, in which physicians have so commonly believed, practically illustrates the same phenomenon. A further obvious requirement is that the quantities of fat used shall be sufficient for the purpose. The smallest quantities are effective in the severest cases, in which the difference between fasting and "basal" or much less than "basal" rations is easily demonstrable. With diminishing severity, the tolerance for fat, like that for carbohydrate, is higher, and correspondingly larger quantities must be given in order to obtain results within a reasonable period of observation.\* Different authors prefer different tests of tolerance. For some, the pro-

\* Objections which seem to have been raised in some quarters against the sudden giving and withdrawing of fat may be answered as follows:

(1) The sudden giving and withdrawal of carbohydrate is a standard method of testing tolerance or measuring the insulin requirement for carbohydrate assimilation. The only difference seems to consist in prejudices in favor of the results shown by such carbohydrate tests and against the results shown by the fat tests.

(2) Digestive disturbances have not arisen from any of these experiments. There is no known basis for the suspicion that such disturbances, if they existed, could alter the diabetic tolerance, unless through impaired absorption of food. But deficient absorption should tend to reduce the metabolic effects, and therefore the effects obtained must be all the more impressive.

(3) If any occult disturbances of digestion or anything else arise from the sudden giving of fat, it is hard to see how such objections can apply to the sudden withdrawal of fat, which is shown to clear up an existing glycosuria, even when the latter has been caused by the addition of carbohydrate.

(4) It is not true that sudden changes are responsible for the results. On the contrary, it has been necessary repeatedly to emphasize that the effects of fat are generally slow and gradual, and the experiments deal mostly with these delayed effects of diets which are continued through weeks or months without discomfort to the patients.

(5) These experiments with insulin are entirely similar in plan and results to those upon which the undernutrition treatment was founded, before insulin was discovered. If any persons have been harboring objections to these previous experiments because of the suddenness of the diet changes, they have overlooked the control experiment which was performed to cover this very point. (Rockefeller Institute Monograph No. 11, p. 522, Table X.) In that experiment, the fat was added as gradually as could well be asked, and the results were entirely typical.



TABLE 1  
Case No. 1044

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Aug.									
17	35	23	0	350	0	.....	166	...	1
18	"	"	"	"	0	.....	209	...	1
19	"	"	"	"	0	5.68	187	137	1
20	"	"	"	"	0	7.44	...	...	1
21	"	"	"	"	0	6.62	180	...	1
22	"	"	"	"	0	7.43	...	...	1
23	"	"	"	"	0	8.00	168	135	1
24	35	14	20	350	0	9.08	168	...	1
25	"	"	"	"	0	7.55	175	134	1
26	"	"	"	"	0	9.19	160	...	1
27	"	"	"	"	0	10.06	178	...	1
28	"	"	"	"	0	6.80	...	132	1
29	"	"	"	"	0	7.52	171	130	1
30	35	200	0	1940	0	8.85	177	128	1
31	"	"	"	"	0	8.01	...	...	1
Sept.									
1	"	"	"	"	0	7.01	203	...	1
2	"	"	"	"	0	9.70	...	...	1
3	"	"	"	"	0	10.00	206	...	1
4	"	"	"	"	0	5.27	...	...	1
5	"	"	"	"	0	4.47	287	126	1
6	"	"	"	"	0	7.17	...	...	1
7	35	0	20	220	0	10.92	232	...	1
8	"	"	"	"	0	6.97	...	...	1
9	"	"	"	"	0	6.26	175	...	1
10	"	"	"	"	0	6.52	...	126	1
11	"	"	"	"	0	6.59	156	...	1
12	"	"	"	"	0	7.65	...	126	1
13	"	"	"	"	0	8.49	...	...	1
14	"	"	"	"	0	4.49	152	129	1
15	"	"	"	"	0	10.91	...	...	1
16	"	"	"	"	0	10.75	...	132	1
17	"	"	"	"	0	...	...	...	1
18	"	"	"	"	0	9.93	132	...	1
19	"	"	"	"	0	11.05	...	...	1
20	"	"	"	"	0	13.34	...	...	1
21	35	150	5	1510	0	13.43	168	127	1
22	"	"	"	"	0	8.18	...	...	1
23	"	"	"	"	0	10.57	...	...	1
24	"	"	"	"	0	10.51	...	...	1
25	"	"	"	"	0	10.00	...	125	1
26	"	"	"	"	0	6.45	200	...	1
27	"	"	"	"	0	10.12	...	...	1
28	"	"	"	"	0	11.12	...	...	1
29	"	"	"	"	0	8.08	...	...	1
30	"	"	"	"	0	7.48	211	...	1
Oct.									
1	"	"	"	"	0	10.04	...	...	4
2	"	"	"	"	0	7.76	...	128	4
3	"	"	"	"	0	6.86	250	...	4
4	"	"	"	"	0	11.65	...	...	4
5	"	"	"	"	0	8.61	...	...	4
6	"	"	"	"	0	8.68	220	125	4

TABLE 1.—*Continued*

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Oct.									
7	35	150	5	1510	0	.....	...	...	4
8	"	"	"	"	0	7.88	...	...	4
9	"	"	"	"	0	7.23	...	126	4
10	"	"	"	"	0	4.59	...	...	4
11	"	"	"	"	0	9.72	226	128	6
12	"	"	"	"	0	10.61	...	...	6
13	"	"	"	"	0	7.34	...	130	6
14	"	"	"	"	0	5.43	...	...	6
15	"	"	"	"	0	6.38	217	...	6
16	"	"	"	"	0	7.42	...	...	6
17	"	"	"	"	0	10.47	...	130	6
18	"	"	"	"	0	7.05	...	...	6
19	"	"	"	"	0	9.05	234	131	6
20	"	"	"	"	0	9.69	...	...	6
21	"	"	"	"	0	2.87	...	...	6
22	"	"	"	"	0	.....	156	...	6

duction of hyperglycemia or the prevention of hypoglycemia by fat will suffice. For others, the appearance of glycosuria in a previously aglycosuric patient will be the preferred test. Others use cases with continued glycosuria, and measure the influence of diet by quantitative variations in the sugar excretion. The admission that this excretion may fluctuate from day to day on account of factors such as the nervous state, which are accidental and not specifically connected with the diabetes, stamps this method as somewhat crude and inaccurate in comparison with the others, which are not subject to these errors. The strongest condemnation of this method is found in the historical fact that the harmfulness of fat remained entirely unperceived as long as physicians used diets which kept up continuous glycosuria in severe cases. Nevertheless, the effect of fat is sufficiently powerful that it can be demonstrated by any of these methods under suitable conditions, and all of them have been used in the present study.

It will be observed that these experiments, like the previous ones with diet alone, are planned so that the results are definite without the need of respiration tests. The utmost that such tests could show would be that the materials metabolized are not identical with those fed, and this rudimentary fact is recognized in

advance. When a semi-starvation regime is imposed, it is obvious that the patient is burning body tissue to supplement his scanty food. When *luxus* rations are given, it is equally obvious that part of the material (particularly fat) is not burned, because the body weight increases. Attention is repeatedly called in the protocols to the fact that these circumstances make the results all the more striking. The benefit of low diets is obtained, even though the metabolism is not as low as the diet. The harm of high diets is demonstrable, even though not all the food is burned and perhaps for the very reason that it is not burned. If respiration studies can throw any light on the reasons for these phenomena, they will be instructive; but the phenomena themselves are neither dependent on respiratory analyses, nor contradictory to any that may be performed.

As most of these cases have been used for several different tests, a logical order of presentation is impossible without breaking up individual records. Any disadvantages of this disorder are believed to be overbalanced by the comparisons thus afforded between the effects of different foods in the same individuals. For the same reason there is some overlapping with Sherrill's work, so that some observations on fat will be found in his paper and some on carbohydrate and protein in this paper. Several incidental illustrations of the influence of fat, total calories and body weight were given in case histories in paper No. 1 (Cases 3, 518, 529, 787, 823, 839, 878, 918, 1016, 1020, 1135, 1167, 1172, 1202, 1244, 1286, 1304, 1332).

Further observations made under experimental conditions are shown in the following tables.

#### REMARKS ON TABLE 1.

With only 1 unit of insulin daily, the patient received a carbohydrate-free diet of 35 gm. protein and 350 calories from Aug. 17 to 23. Aug. 24 to 29, 20 gm. carbohydrate was introduced, with a corresponding reduction of fat so as to keep the total calories at 350. The table shows that neither glycosuria nor any important elevation of plasma sugar resulted.

From Aug. 30 to Sept. 6, the carbohydrate was withdrawn and the supposed glucose equivalent given in the form of 200 gm. of fat. This form of test is obviously unfair, because no allowance is made for the quantities of body fat which were necessarily burned with the previous diet. Notwithstanding this handicap, an effect is very evident in the rise of plasma sugar, though the test was not continued long enough for the production of glycosuria.

From Sept. 7 to 20, the 200 gm. of fat was replaced by 20 gm. of carbohydrate, making a fat-free diet of only 220 calories instead of the

preceding 1940 calories. The plasma sugar gradually fell, showing the well known effect of undernutrition, notwithstanding the quantities of body fat that must have entered into the metabolism at this time.

TABLE 2  
Case No. 1135

Date 1922	DIET				Qualita- tive Glucose	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Nov.								
24	40	46	5	600	++	184	64	..
25	"	"	"	"	0	...	...	...
26	"	"	"	"	0	200	61	...
27	"	"	"	"	0	...	...	...
28	"	"	"	"	0	...	61	...
29	"	"	"	"	0	195	...	...
30	"	"	"	"	0	...	65	2
Dec.								
1	"	"	"	"	0	142	...	2
2	"	"	"	"	0	...	64	2
3	"	"	"	"	0	...	...	2
4	"	"	"	"	0	142	66	2
5	"	"	"	"	0	...	...	2
6	40	46	25	680	0	150	67	2
7	"	"	"	"	0	...	...	2
8	"	"	"	"	0	...	67	2
9	"	"	"	"	0	146	...	2
10	"	"	"	"	0	...	67	0
11	"	"	"	"	0	...	...	2
12	"	"	"	"	0	...	67	2
13	"	"	"	"	0	...	...	2
14	"	"	"	"	0	169	67	2
15	"	"	"	"	0	...	...	2
16	"	"	"	"	0	...	65	2
17	40	200	5	1980	0	250	...	2
18	"	"	"	"	0	...	66	2
19	"	"	"	"	0	...	...	2
20	"	"	"	"	0	254	61	2
21	"	"	"	"	0	306	...	2
22	"	"	"	"	0	...	66	2
23	"	"	"	"	+	...	...	2
24	40	1	25	269	+	441	66	2
25	"	"	"	"	+	...	...	2
26	"	"	"	"	0	300	65	2
27	"	"	"	"	0	250	...	2
28	"	"	"	"	0	...	66	2
29	"	"	"	"	0	272	...	2
30	"	"	"	"	0	...	66	2 1/2
31	"	"	"	"	0	...	...	5
1923								
Jan.								
1	"	"	"	"	0	...	66	5
2	"	"	"	"	0	242	...	5
3	"	"	"	"	0	...	64	5
4	"	"	"	"	0	211	...	5

TABLE 3  
Case No. 823

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Jan.								
19	55	20	100	800	52.80	518	86	10
20	"	"	"	"	38.70	319	...	10
21	"	"	"	"	29.68	263	88	10
22	"	"	"	"	37.41	375	...	10
23	"	"	"	"	29.40	268	88	10
24	"	"	"	"	76.68	300	...	10
25	"	"	"	"	60.08	334	89	10
26	"	"	"	"	65.91 $\frac{1}{2}$	357	...	10
27	"	"	"	"	77.10 $\frac{1}{2}$	349	90	10
28	"	"	"	"	64.19	...	...	15
29	"	"	"	"	54.33	...	88	15
30	"	"	"	"	57.61	300	...	15
31	"	"	"	"	86.85 $\frac{1}{2}$	...	88	15
Feb.								
1	"	"	"	"	66.60	392	...	15
2	55	42	50	800	53.71	...	88	15
3	"	"	"	"	54.80	366	...	15
4	"	"	"	"	86.00	...	88	15
5	"	"	"	"	46.20	294	...	15
6	"	"	"	"	35.70	...	88	15
7	"	"	"	"	60.72	272	...	15
8	"	"	"	"	89.26	326	89	15
9	"	"	"	"	39.48	...	...	15
10	"	"	"	"	28.08	...	89	15
11	"	"	"	"	17.10	...	...	21
12	"	"	"	"	30.40	288	89	21
13	"	"	"	"	13.12	...	...	21
14	"	"	"	"	3.68	250	91	21
15-16	"	"	"	"	0	...	92	21
17	55	175	50	2000	0	193	...	21
18	"	"	"	"	+	...	96	24
19	"	"	"	"	+++	407	...	24
20	"	"	"	"	++++	...	96	24
21	"	"	"	"	81.07	...	...	27
22	"	"	"	"	80.85	349	98	27
23	"	"	"	"	63.64	...	...	27
24	"	"	"	"	40.04	...	98	27
25	"	"	"	"	31.61	...	...	30
26	"	"	"	"	63.00	416	100	30
27	"	"	"	"	37.76	...	...	30
28	"	"	"	"	71.28	...	...	30
Mar.								
1	"	"	"	"	79.00	416	101	36
2	"	"	"	"	36.86	...	...	36
3	"	"	"	"	64.24	...	102	36
4	"	"	"	"	27.03	...	102	40
5	"	"	"	"	8.13	...	...	40
6	"	"	"	"	57.67	319	103	40
7	"	"	"	"	25.85	...	...	40
8	"	"	"	"	5.32	405	103	45
9	"	"	"	"	0	...	...	45
10	"	"	"	"	0	90*	104	45

\*Blood sample taken at 8 p. m. Other blood samples taken before breakfast.

TABLE 3.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
11	55	131	150	2000	15.34	65*	...	45
12	"	"	"	"	0	312	108	45
13	"	"	"	"	0	...	...	45
14	"	"	"	"	0	...	108	45
15	"	"	"	"	0	62*	...	45
16	"	"	"	"	0	...	109	45
17	"	"	"	"	0	...	...	45
18	"	"	"	"	0	...	110	45
19	"	"	"	"	0	169*	...	45
20	"	"	"	"	0	...	109	45
21	"	"	"	"	0	...	...	45
22	"	"	"	"	0	305	110	45
23	"	"	"	"	4.86	...	...	45
24	"	"	"	"	31.20	119*	111	45
25	55	175	50	2000	11.16	...	...	45
26	"	"	"	"	+	312	111	45
27	"	"	"	"	0	159	...	45
28	"	"	"	"	0	99*	113	45
29	"	"	"	"	0	...	...	45
30	"	"	"	"	0	234	114	45
31	"	"	"	"	0	79*	115	45
April								
1	"	"	"	"	0	...	114	45
2	"	"	"	"	0	...	...	45
3	"	"	"	"	0	211	114	45
4	"	"	"	"	0	...	...	45
5	"	"	"	"	0	...	115	45
6	"	"	"	"	0	68*	...	45
7	"	"	"	"	0	...	115	45
8	55	231	50	2500	0	...	...	45
9	"	"	"	"	0	...	116	45
10	"	"	"	"	0	...	...	45
11	"	"	"	"	0	...	115	45
12	"	"	"	"	0	79	...	45
13	"	"	"	"	0	...	117	45
14	"	"	"	"	+	...	...	45
15	"	"	"	"	9.30	...	118	45
16	"	"	"	"	27.00	...	...	45
17	"	"	"	"	17.40	...	119	45

\*Blood sample taken at 8 p.m. Other blood samples taken before breakfast.

From Sept. 21 to Oct. 22, a different diet with the same supposed glucose equivalent was given, namely 35 gm. protein, 5 gm. carbohydrate, 150 gm. fat and 1510 calories. The plasma sugar rose as usual with the increased calories, and instead of changing the diet the insulin dosage was increased. Up to Oct. 11, 4 units failed to keep the plasma sugar below 0.2 per cent. An increase to 6 units then gradually restored the plasma sugar to approximately the level which it had held with the low-calory diets.



The entire experiment was conducted without glycosuria. If the plasma sugar be accepted as a reliable guide, or if it be admitted that the increases of plasma sugar would have led to glycosuria if the high calory diets had been continued longer, it can be concluded that the insulin requirement for the high calory diets was approximately six times as great as for low calory diets of the same theoretical glucose value, even with no allowance for the burning of body fat.

#### REMARKS ON TABLE 2.

From Nov. 30 to Dec. 5, insulin in dosage of 2 units daily kept glycosuria absent and the plasma sugar only slightly above normal on a diet of 40 gm. protein, 5 gm. carbohydrate and 600 calories. Beginning Dec. 6, 20 gm. carbohydrate was added, making 25 gm. carbohydrate and 680 calories in the diet. The plasma sugar thus rose to 0.250 per cent. on the morning of Dec. 17, and it is conceivable that glycosuria might have resulted from continuance of this diet. Beginning Dec. 17, however, the fat was sharply increased, so as to make the diet 40 gm. protein, 5 gm. carbohydrate and 1980 calories. The glucose value of the diet was thus, according to Woodyatt's calculation, slightly less than before. The result was that the hyperglycemia continued to rise and glycosuria appeared on Dec. 23.

In order to determine the relative responsibility of sugar and fat, the protein was kept unchanged, the carbohydrate increased to 25 gm., and fat almost completely excluded, thus making a diet of only 269 calories. Glycosuria ceased within 3 days and hyperglycemia diminished, illustrating the well known influence of undernutrition, though the glucose value of the diet had not been reduced and the patient in addition was compelled to burn body fat and probably body protein. From Dec. 24 to 29, hyperglycemia was reduced in this way from 0.441 to 0.272 per cent.

Beginning Dec. 30, the insulin dosage was increased. There is the usual illustration of the extra pancreatic labor represented by hyperglycemia, since 5 units of insulin from Dec. 31 to Jan. 4 failed to lower the plasma sugar to any great extent.

#### REMARKS ON TABLE 3.

On the diet of 55 gm. protein, 100 gm. carbohydrate and 800 calories from Jan. 19 to Feb. 1, heavy glycosuria continued and was not controlled by increase of insulin up to 15 units daily. The carbohydrate was then reduced to 50 gm. and a corresponding increase of fat given, so as to keep the total calories constant at 800. Glycosuria scarcely diminished, and was not abolished until the insulin dosage was increased to 21 units, Feb. 11-16.

Beginning Feb. 17, the protein and carbohydrate were continued unchanged, but the fat was increased so as to raise the total calories to 2000 per day. Glycosuria appeared on the second day, and was not controlled until insulin was increased to 45 units per day, March 8-10. The influence of fat in causing glycosuria and raising the insulin requirement is thus seen to be out of all proportion to any supposed carbohydrate content of the fat.



Beginning March 11, the carbohydrate was raised to 150 gm., and fat was reduced so as to keep the total calories constant at 2000 per day. Glycosuria remained absent until March 23-25, and then was moderate in quantity. The carbohydrate was then reduced to 50 gm. daily and fat was

TABLE 4  
Case No. 229

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	CaL.				
Oct.								
2	15	0	15	120	.....	...	36	..
3	"	"	"	"	++++	359	...	2
4	"	"	"	"	++	...	...	2
5	"	"	"	"	+	...	38	2
6	"	"	"	"	+	206	...	2
7	"	"	"	"	0	...	39	2
8	"	"	"	"	0	168	...	2
9	"	"	"	"	0	...	39	3
10	"	"	"	"	0	123	...	3
11	30	28	5	400	0	...	38	3
12	"	"	"	"	0	...	...	3
13	"	"	"	"	+	429	...	3
14	"	"	"	"	+	...	40	3
15	"	"	"	"	+	385	...	3
16	"	"	"	"	+	...	...	5
17	"	"	"	"	+	385	38	5
18	"	"	"	"	++	...	...	5
19	"	"	"	"	++++	429	...	0
20	"	"	"	"	++++	...	39	2 $\frac{1}{2}$
21	"	"	"	"	++	...	...	5
22	"	"	"	"	++	318	...	5
23	"	"	"	"	+	357	...	5
24	"	"	"	"	+	405	...	5
25	"	"	"	"	+	...	...	5
26	"	"	"	"	0	...	...	5
27	"	"	"	"	0	...	37	5
28	"	"	"	"	0	...	...	5
29	"	"	"	"	0	270	36	8
30	"	"	"	"	+	...	...	8
31	"	"	"	"	0	...	38	8
Nov.								
1	"	"	"	"	0	263	...	8
2	40	44	10	600	0	...	37	8
3	"	"	"	"	0	...	...	8
4	"	"	"	"	+	326	35	8
5	"	"	"	"	++	...	...	12
6	"	"	"	"	0	...	35	14

increased so as to keep the total calories at 2000. Glycosuria promptly ceased and the plasma sugar fell with the same dosage of insulin.

A further increase of fat to make 2500 total calories, beginning April 8, resulted in a return of glycosuria with the same insulin dosage, as shown in the table.

TABLE 5  
Case No. 574

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insu- lin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Aug.									
24	60	60	6	804	0	.....	...	18	2
25	"	"	"	"	0	.....	154	..	2
26	"	"	"	"	0	.....	...	18	2
27	"	"	"	"	0	8.30	...	..	2
28	"	"	"	"	0	4.42	...	18	2
29	"	"	"	"	0	4.23	...	..	2
30	"	"	"	"	0	3.53	173	20	2
31	"	"	"	"	0	4.67	...	..	0
Sept.									
1	"	"	"	"	0	5.47	159	20	2
2	"	"	"	"	0	8.41	140	..	2
3	"	"	"	"	0	5.88	...	21	2
4	"	"	"	"	0	6.97	...	20	2
5	"	"	"	"	0	7.06	...	..	2
6	"	"	"	"	0.89	6.65	230	..	2
7	"	"	"	"	5.69	7.50	...	21	2
8	"	"	"	"	7.27	7.92	...	..	2
9	60	10	16	394	9.05	8.35	...	21	2
10	"	"	"	"	2.29	6.39	...	..	2
11	"	"	"	"	0	5.57	...	22	2
12	"	"	"	"	0	5.11	...	..	2
13	"	"	"	"	0	3.61	...	21	2
14	"	"	"	"	0	6.52	...	..	2
15	"	"	"	"	0	4.45	...	22	2
16	"	"	"	"	0	8.48	...	..	2
17	"	"	"	"	0	3.95	...	21	2
18	"	"	"	"	0	7.89	...	..	2
19	"	"	"	"	0	8.57	...	22	2
20	"	"	"	"	0	6.56	...	..	2
21	"	"	"	"	0	7.23	...	21	2
22	"	"	"	"	0	7.15	...	..	2
23	"	"	"	"	0	9.35	126	22	2
24	60	60	6	804	0	5.90	133	..	2
25	"	"	"	"	1.72	7.78	...	20	2
26	"	"	"	"	3.72	5.42	...	..	2
27	"	"	"	"	8.24	7.59	...	20	2
28	"	"	"	"	3.57	4.99	...	..	2
29	"	"	"	"	5.02	4.29	...	20	2
30	"	"	"	"	6.23	5.93	...	..	2
Oct.									
1	"	"	"	"	8.72	12.15	...	21	2
2	"	"	"	"	10.15	6.20	...	..	2
3	60	10	11	374	7.05	4.89	254	21	2
4	"	"	"	"	5.83	7.38	...	..	2
5	"	"	"	"	0	6.65	...	21	2
6	"	"	"	"	0	7.09	...	..	2
7	"	"	"	"	0	7.38	...	19	2
8	"	"	"	"	0	5.98	...	..	2
9	"	"	"	"	0	7.15	...	20	2
10	"	"	"	"	0	4.81	...	..	2
11	"	"	"	"	0	7.05	...	20	2
12	"	"	"	"	0	8.30	...	..	2
13	"	"	"	"	0	4.97	...	21	2

TABLE 5.—Continued

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insu- lin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
14	60	10	11	374	0	5.17	159	..	2
15	"	"	"	"	0	.....	...	20	2
16	"	"	"	"	0	4.67	...	..	2
17	"	"	"	"	0	5.64	...	21	2
18	"	"	"	"	0	6.57	...	..	2
19	"	"	"	"	0	6.11	100	20	2
20	"	"	"	"	0	.....	...	..	2
21	"	"	"	"	0	.....	...	..	2
22	"	"	"	"	0	6.63	220	..	2
23	"	"	"	"	0	6.66	175	21	2
24	"	"	"	"	0	7.32	152	..	2
25	"	"	"	"	0	5.47	...	20	2
26	"	"	"	"	0	5.68	...	..	2
27	"	"	"	"	0	5.71	...	20	2
28	"	"	"	"	0	8.39	125	..	2
29	"	"	"	"	0	7.81	...	20	2
30	"	"	"	"	0	6.86	...	..	2
31	"	"	"	"	0	3.27	...	20	2
Nov.									
1	"	"	"	"	0	4.52	152	..	2
2-3	60	60	6	804	0	6.40	...	20	2
4	"	"	"	"	0	7.06	125	20	2
5-6	"	"	"	"	0	7.30	...	21	2
7	"	"	"	"	0	5.71	156	..	2
8-9	60	60	20	860	0	6.22	...	20	2
10	"	"	"	"	0	8.72	195	20	2
11	"	"	"	"	0	6.78	...	..	2
12	"	"	"	"	+	4.50	220	21	2
13	"	"	"	"	+	6.72	...	..	2
14	"	"	"	"	++++	7.66	270	21	2
15-16	"	"	"	"	+++	7.56	...	20	4
17	"	"	"	"	++	6.38	357	..	4
18	"	"	"	"	++++	6.66	...	..	4
19-20	"	"	"	"	++++	8.80	...	20	6
21	"	"	"	"	13.57	5.93	...	..	6
22	"	"	"	"	26.94	8.89	...	21	4
23	"	"	"	"	22.46	7.01	...	..	6
24	"	"	"	"	13.72	8.08	...	21	6
25	"	"	"	"	14.67	8.33	...	..	6
26	"	"	"	"	++	6.69	...	21	6
27-28	"	"	"	"	0	7.53	...	22	6
29	"	"	"	"	+	6.13	...	..	3
30	"	"	"	"	+	8.34	.....	21	6
Dec.									
1	"	"	"	"	0	.....	223	..	6
2-7	"	"	"	"	0	8.40	...	22	6
8	"	"	"	"	0	8.54	223	22	6
9	"	"	"	"	0	7.86	...	..	6
10	"	"	"	"	0	8.27	...	23	2
11	"	"	"	"	0	8.95	...	..	4
12-15	"	"	"	"	0	8.05	...	22	6
16	"	"	"	"	0	4.65	146	23	6
17-18	"	"	"	"	0	8.32	...	23	6
19	"	"	"	"	0	9.01	156	..	6
20-22	"	"	"	"	0	8.30	...	23	6
23-27	"	"	"	"	0	.....	...	..	6

TABLE 5.—Continued

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insu- lin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
28	60	60	20	860	0	9.36	...	24	6
29	"	"	"	"	0	7.95	130	..	6
30-31	"	"	"	"	0	7.76	...	21	6
1923									
Jan.									
1	"	"	"	"	0	9.24	159	24	6
2	"	"	"	"	+	6.87	...	..	6
3	"	"	"	"	2.19	8.91	...	24	6
4	"	"	"	"	0	8.88	...	..	6
5	"	"	"	"	0	7.66	246	23	6
6-7	"	"	"	"	0	8.83	...	25	6
8	"	"	"	"	0	...	220	..	..
9-10	"	"	"	"	0	7.48	...	23	6
11	"	"	"	"	+	10.21	...	23	9
12-13	"	"	"	"	++	9.06	...	23	9
14	"	"	"	"	35.70	11.33	300	..	9
15	"	"	"	"	25.56	9.54	...	22	9
16	"	"	"	"	22.42	9.11	...	..	9
17	"	"	"	"	29.38	9.64	...	24	9
18	"	"	"	"	26.46	9.01	208	..	12
19	"	"	"	"	28.84	8.62	...	24	12
20	"	"	"	"	9.87	7.41	...	..	12
21	"	"	"	"	22.12	8.92	357	23	12
22	"	"	"	"	18.98	9.44	...	..	12
23	"	"	"	"	12.48	8.87	...	24	12
24	"	"	"	"	3.50	8.51	...	..	12
25	"	"	"	"	7.75	9.66	405	24	12
26	"	"	"	"	5.28	10.08	...	..	12
27	"	"	"	"	3.22	10.19	405	25	12
28-29	"	"	"	"	0	9.02	...	24	12
30	"	"	"	"	+	9.47	...	..	12
31	"	"	"	"	0	6.76	230	25	12
Feb.									
1	"	"	"	"	0	5.22	...	..	12
2	"	"	"	"	0	8.06	230	25	12
3-4	"	"	"	"	0	9.24	...	25	12
5	"	"	"	"	0	12.00	187	..	12
6-7	"	"	"	"	0	9.20	...	25	12
8	"	"	"	"	0	8.60	203	26	12
9-10	"	"	"	"	0	9.89	...	26	12
11-13	"	"	"	"	0	...	...	26	12
14	"	"	"	"	0	...	214	26	12
15-18	"	"	"	"	0	...	...	26	12
19	"	"	"	"	++	...	357	..	12
20	"	"	"	"	14.69	...	...	..	12
21	60	42	60	860	9.60	...	...	26	12
22	"	"	"	"	17.68	...	...	..	12
23	"	"	"	"	9.30	...	...	26	12
24	"	"	"	"	11.52	...	341	..	12
25	"	"	"	"	4.90	...	...	26	12
26	"	"	"	"	4.08	...	...	..	12
27	"	"	"	"	+	...	...	27	12
28	"	"	"	"	2.00	...	254	28	12
Mar.									
1	"	"	"	"	0	...	...	..	15
2	"	"	"	"	+	...	270	28	15

TABLE 5.—Continued

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insu- lin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
3	60	42	60	860	12.79	.....	341	..	15
4	"	"	"	"	++++	.....	349	28	15
5	"	"	"	"	24.00	.....	270	..	10
6	"	"	"	"	++++	.....	.....	28	15
7	"	"	"	"	3.77	.....	.....	..	15
8	"	"	"	"	0	.....	.....	28	15

The entire experiment proves that fat has a powerful influence in causing glycosuria and raising the insulin requirement, but is less powerful in this respect than the caloric equivalent of carbohydrate, at least during experimental periods of the length used here.

## REMARKS ON TABLE 4.

This child became free from glycosuria and the plasma sugar fell to the low level of 0.123 per cent. on a fat-free diet of 15 gm. protein and 15 gm. carbohydrate up to Oct.<sup>o</sup> 10, with only 2 or 3 units of insulin per day. On Oct. 11 the diet was changed to 30 gm. protein, 5 gm. carbohydrate, and 28 gm. fat, making 400 calories. Within 2 days hyperglycemia of 0.429 per cent. and glycosuria were present. The new ration had no higher glucose value than the first one, if any allowance be made at all for the quantities of body fat and probably of body protein that were necessarily burned with the first diet.

It proved necessary to increase the insulin gradually to 8 units per day (October 29—November 1) in order to stop glycosuria on the 400 calory diet, and even this dosage did not restore the former normal level of blood sugar. The original diet of 120 calories was not as radical as complete fasting. The second diet of 400 calories conforms somewhat to the ideas of Newburgh and also of writers who have imagined that a "basal" diet is as efficacious as fasting. This severe case afforded the opportunity of proving that the insulin requirement with this latter type of diet was more than four times as great as with fasting.

The subsequent increase to 40 gm. protein, 10 gm. carbohydrate and 600 calories was not planned as part of the experiment, but illustrates the increase of insulin requirement to 14 units per day with this higher diet. Our general information makes it highly improbable that the extra 6 units were required merely for the increase of 10 gm. protein and 5 gm. carbohydrate, and gives reason to believe that the increase of fat and total calories was the most important factor.

## REMARKS ON TABLE 5.

This child received 2 units of insulin daily, and from Aug. 24 to Sept. 8 the diet was 60 gm. protein, 60 gm. fat, 6 gm. carbohydrate and 804

TABLE 6  
Case No. 878

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Nov.								
4	55	137	10	1500	0	...	71	1½
5	"	"	"	"	0	120	...	1
6	"	"	"	"	0	...	72	1
7	60	164	20	1800	0	...	...	1
8	"	"	"	"	0	...	72	1
9	"	"	"	"	0	...	...	1
10	"	"	"	"	0	135	73	1
11	"	"	"	"	0	...	...	1
12	"	"	"	"	0	161	73	1
13	"	"	"	"	0	...	...	2
14	"	"	"	"	0	171	72	2
15	"	"	"	"	0	...	...	2
16	"	"	"	"	0	...	74	4
17	"	"	"	"	0	...	...	4
18	"	"	"	"	0	...	73	4
19	"	"	"	"	0	...	...	6
20	"	"	"	"	0	...	73	6
21	"	"	"	"	0	...	...	6
22	"	"	"	"	0	184	74	6
23	60	164	50	1920	0	...	...	6
24	"	"	"	"	0	217	73	6
25	"	"	"	"	0	...	...	6
26	"	"	"	"	0	...	73	6
27	"	"	"	"	0	...	...	6
28	"	"	"	"	0	206	75	3
29	"	"	"	"	0	...	...	3
30	"	"	"	"	0	...	75	6
Dec.								
1	"	"	"	"	0	...	...	6
2	"	"	"	"	0	230	75	6
3	"	"	"	"	0	...	...	6
4	"	"	"	"	0	...	76	6
5	"	"	"	"	0	...	...	6
6	"	"	"	"	0	203	76	3
7	"	"	"	"	0	...	...	6
8	"	"	"	"	0	...	76	6
9	"	"	"	"	0	199	...	6
10	"	"	"	"	+	...	76	0
11	"	"	"	"	+	...	...	3
12	"	"	"	"	+	...	76	6
13	"	"	"	"	0	227	...	6
14	"	"	"	"	0	...	76	6
15	"	"	"	"	0	...	...	6
16	"	"	"	"	0	...	76	6
17	"	"	"	"	0	294	...	8
18	"	"	"	"	0	...	76	8
19	"	"	"	"	0	...	...	8
20	"	"	"	"	0	...	77	8
21	"	"	"	"	0	...	...	4
22	"	"	"	"	0	272	77	8
23	60	133	40	1600	0	...	...	8
24	"	"	"	"	0	...	...	8
25	"	"	"	"	0	...	...	8

TABLE 6.—Continued

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
26	60	133	40	1600	0	...	...	8
27	"	"	"	"	0	...	...	8
28	"	"	"	"	0	272	77	8
29	"	"	"	"	0	226	...	8
30	"	"	"	"	+	...	76	8
31	"	"	"	"	+	...	...	8
1923								
Jan.								
1	"	"	"	"	+	...	76	8
2	"	"	"	"	+	...	...	8
3	"	"	"	"	+	263	76	8
4	"	"	"	"	+	...	...	8
5	"	"	"	"	0	...	77	8
6	"	"	"	"	0	...	...	8
7	"	"	"	"	0	...	76	8
8	"	"	"	"	0	...	...	8
9	"	"	"	"	3.58	...	75	8
10	"	"	"	"	5.51	263	...	8
11	"	"	"	"	0	...	77	12
12	"	"	"	"	8.12	272	...	12
13	"	"	"	"	0	...	77	12
14	"	"	"	"	0	230	...	12
15	"	"	"	"	0	...	76	12
16	"	"	"	"	0	...	...	12
17	"	"	"	"	0	...	77	12
18	"	"	"	"	0	195	...	12
19	"	"	"	"	0	...	77	12
20	"	"	"	"	0	...	...	12
21	60	177	40	2000	10.56	220	75	12
22	"	"	"	"	29.82	...	...	12
23	"	"	"	"	29.60	268	76	12
24	"	"	"	"	22.00	...	...	12
25	"	"	"	"	21.0	306	76	12
26	"	"	"	"	31.68	...	...	12
27	"	"	"	"	40.32	306	76	12
28	"	"	"	"	38.49	...	...	12
29	"	"	"	"	21.0	...	71	18
30	"	"	"	"	44.0	294	...	18
31	"	"	"	"	39.60	...	77	18
Feb.								
1	"	"	"	"	33.9	...	...	18
2	"	"	"	"	27.50	...	77	18
3	"	"	"	"	0	...	...	18
4	"	"	"	"	23.40	334	78	24
5	"	"	"	"	16.65	...	...	24
6	"	"	"	"	16.0	...	78	24
7	"	"	"	"	0	270	...	24
8	"	"	"	"	0	...	75	30
9	"	"	"	"	0	...	...	30
10	"	"	"	"	0	246	79	30
11	"	"	"	"	0	...	...	30
12	"	"	"	"	0	...	79	30
13	"	"	"	"	0	203	...	30
14	"	"	"	"	0	...	80	30
15	"	"	"	"	...	234	...	30
16	60	151	100	2000	0	...	80	30



TABLE 6.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.								
17	60	151	100	2000	2.25	...	...	30
18	"	"	"	"	0	...	80	30
19	"	"	"	"	0	217	...	30
20	"	"	"	"	0	...	80	30
21	"	"	"	"	0	...	...	30
22	"	"	"	"	0	...	81	30
23	"	"	"	"	0	...	...	30
24	"	"	"	"	0	195	81	30
25	"	"	"	"	0	...	...	30
26	"	"	"	"	0	...	81	30
27	"	"	"	"	0	200	...	30
28	"	"	"	"	0	...	82	30
Mar.								
1	"	"	"	"	0	...	...	30
2	"	"	"	"	0	...	82	30
3	"	"	"	"	0	...	...	30
4	120	151	200	2640	18.40	...	82	30
5	"	"	"	"	2.47	...	...	30
6	"	"	"	"	12.42	226	83	30
7	"	"	"	"	5.20	...	...	30
8	"	"	"	"	24.45	270	83	30
9	"	"	"	"	19.52	...	...	30
10	"	"	"	"	12.61	...	84	30
11	"	"	"	"	29.70	272	...	30
12	"	"	"	"	17.28	...	86	30
13	"	"	"	"	...	...	...	30
14	"	"	"	"	7.25	...	86	36
15	"	"	"	"	30.40	...	...	36
16	"	"	"	"	11.68	...	87	36
17	"	"	"	"	7.85	300	...	36
18	"	"	"	"	15.00	...	88	36
19	"	"	"	"	+	254	...	36
20	"	"	"	"	10.54	...	87	36
21	"	"	"	"	11.78	...	...	36
22	"	"	"	"	13.60	242	89	36
23	"	"	"	"	10.57	...	...	36
24	"	"	"	"	6.14	...	90	36
25	"	"	"	"	6.00	...	...	40
26	"	"	"	"	4.80	254	90	40
27	"	"	"	"	7.80	254	...	40
28	"	"	"	"	8.90	...	92	42
29	"	"	"	"	9.90	...	...	42
30	"	"	"	"	12.10	272	92	42
31	"	"	"	"	...	...	...	42
April								
1	"	"	"	"	10.40	...	92	45
2	"	"	"	"	0	234	...	45
3	"	"	"	"	1.70	...	94	45
4	"	"	"	"	5.00	...	...	45
5	"	"	"	"	0	234	92	45
6	"	"	"	"	...	...	...	45
7	"	"	"	"	...	...	92	45
8	"	"	"	"	...	...	...	45
9	"	"	"	"	0	...	...	45
10	"	"	"	"	8.00	...	...	45
11	"	"	"	"	0	...	96	45

TABLE 6.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
April								
12	120	151	200	2640	3.10	230	...	45
13	"	"	"	"	3.20	...	97	45
14	"	"	"	"	11.20	...	...	45
15	"	"	"	"	+	...	98	48
16	"	"	"	"	.....	230	...	48

calories. The plasma sugar gradually rose, and from Sept. 6 to 8 there was increasing glycosuria.

A change was then made to a lower diet with slightly higher glucose value, viz. 60 gm. protein, 10 gm. fat, 16 gm. carbohydrate and 394 calories. Glycosuria ceased within 2 days, and by Sept. 23 the plasma sugar had fallen to the approximately normal level of 0.126 per cent.

Sept. 24 to Oct. 2, the former diet of 60 gm. protein, 6 gm. carbohydrate and 804 calories was resumed. Glycosuria returned with unusual promptness, and increased up to the end of this period.

Oct. 3 to Nov. 1, a lower diet with the identical glucose value was given, namely 60 gm. protein, 10 gm. fat, 11 gm. carbohydrate and 374 calories. Glycosuria ceased quickly and the plasma sugar fell, as usual with undernutrition.

Beginning Nov. 2, the diet was increased first to the old ration of 804 calories and then to 60 gm. protein, 20 gm. carbohydrate and 860 calories. This diet continued from Nov. 8 to Feb. 20. Glycosuria began Nov. 12, and no influence of the additional carbohydrate (as compared with the 804 calorie ration) was perceptible in either the promptness of its onset or the quantities of sugar excreted. The insulin dosage was then increased to meet the requirement on this diet. It will be noticed that in December, 6 units per day not only sufficed to abolish glycosuria but also reduced the plasma sugar gradually to 0.130 per cent. The body weight was rising, however, and, as has often been stated, the full effect of fat is frequently slow in appearing. Accordingly, there was an outbreak of heavy glycosuria in January, and 12 units of insulin finally proved inadequate to keep the urine free from sugar.

Beginning Feb. 21, the glucose intake was increased without altering the total calories, by changing the diet to 60 gm. protein, 60 gm. carbohydrate, 42 gm. fat and 860 calories. There is no indication that the glycosuria or hyperglycemia was affected by this change. Fifteen units of insulin per day finally controlled the glycosuria. It seems probable that this quantity would have been required by the high calorie diet and increasing body weight, irrespective of the last increase of the carbohydrate allowance.

In summary, this experiment shows the markedly greater tendency to glycosuria with high fat diets, as compared with lower diets of equivalent

glucose value. The pernicious influence of these diets, which was demonstrated during the former treatment of diabetes by diet alone, is thus confirmed. Likewise, the multiplied insulin requirement with the high fat diets gives a numerical expression to the increased burden which these diets imposed upon the weakened pancreatic function. All the observations furthermore illustrate the impossibility of reckoning the insulin requirement according to the glucose value of the diet, and the dominant importance of the total calories and body weight.

#### REMARKS ON TABLE 6.

With the diets used at the beginning, which were only slightly above this patient's tolerance, her insulin requirement was low. With the diet of 60 gm. protein, 20 gm. carbohydrate and 1800 calories, however, it became necessary to increase the insulin gradually to 6 units per day up to Nov. 22. November 23–December 22, the carbohydrate was increased to 50 gm. without change in the protein or fat, thus raising the total calories to 1920. This increase of 30 gm. carbohydrate seemed to raise the insulin requirement by about 2 units.

December 23–January 20, both carbohydrate and fat were reduced so as to make the diet 60 gm. protein, 40 gm. carbohydrate and 1600 calories. Nevertheless, glycosuria occurred and it became necessary to increase the insulin dosage to 12 units, in order to keep the patient sugar-free for the 8 days preceding Jan. 21. This observation on the lower diet makes it evident that the preceding calculation of a 2-unit increase of insulin requirement was too low. The extra requirement evidently amounted to more than 6 units, and this result in a prolonged experiment illustrates the danger of drawing conclusions from too brief observations.

January 21–February 15, the diet was raised to 2000 calories by increase of fat. The occurrence of glycosuria on the very first day of this increase is highly exceptional, as the influence of fat is generally much slower. In order to check the glycosuria it became necessary to increase the insulin dosage to 30 units per day. This increased requirement of 18 units of insulin to balance an increase of 44 gm. of fat thus appears greater than the 6 units required to balance the increase of 30 gm. of carbohydrate. The real reason for the great discrepancy was that the diet was now approaching a *luxus* level, so that a considerable increase of body weight was in progress.

February 16–March 3, the carbohydrate was increased to 100 gm., while the fat was reduced so as to keep the total calories unchanged at 2000. Under these circumstances, no glycosuria resulted, and the plasma sugar actually fell a trifle lower than before. This is one of the minority of instances in which no difference in glycosuric effect has been perceptible between equivalent caloric quantities of fat and carbohydrate.

Beginning March 4, both protein and carbohydrate were doubled, while the fat was left unchanged, making the diet 120 gm. protein, 151 gm. fat, 200 gm. carbohydrate, and 2640 calories. Glycosuria appeared immediately, but was small in amount compared with the increase of carbohydrate in the diet. Nevertheless, an increase of insulin dosage to

48 units was necessary in order to make the urine sugar-free. This large increase of insulin requirement may be accounted for by the simultaneous increase of protein, carbohydrate, total calories, and body weight.

#### REMARKS ON TABLE 7.

This 8-year-old girl, when placed on a diet of 60 gm. protein, 30 gm. carbohydrate and 1400 calories, developed hyperglycemia and later glycosuria, notwithstanding a gradual increase of insulin dosage to 7 units per day. Sept. 5 to 24, the fat was reduced by 100 gm. and the carbohydrate increased by 10 gm., so as to keep the theoretical glucose value of the diet unchanged while reducing the total calories to an under-nutrition level of 535. No allowance was made for the body fat and perhaps protein which would be burned under this regime and would increase the amount of glucose entering into the metabolism. On this program the usual effects of undernutrition appeared; glycosuria ceased Sept. 11, and by Sept. 24 the plasma sugar had fallen to 0.137 per cent. though the insulin on the closing days was reduced to 6 units. Sept. 25-Oct. 8, the diet of 60 gm. protein, 30 gm. carbohydrate and 1400 calories was resumed, and the plasma sugar quickly rose. When glycosuria appeared, beginning Sept. 28, it was checked by increasing insulin dosage, and up to Oct. 8 it was evident that 18 units per day were required for this purpose, without reducing the plasma sugar to normal. It thus appeared that 100 gm. of fat increased the insulin requirement by at least 12 units per day.

With a reduction of fat beginning Oct. 9, so as to reduce the total calories to 1200, slight hyperglycemia continued without glycosuria on the same insulin dosage. Withdrawal of insulin for brief periods in the latter part of October resulted in prompt glycosuria. An increase of insulin to 20 units per day was necessary to reduce the plasma sugar nearly to normal from Nov. 5 to 12. The difference between hyperglycemia and normal blood sugar thus amounted to 2 units or more of insulin per day in this instance.

November 13 to Dec. 9, fat was increased so as to raise the total calories first to 1600 and then to 1800, without changing protein or carbohydrate. Hyperglycemia and glycosuria resulted with the same insulin dosage. December 10-13, the carbohydrate was increased to 60 gm. and the fat reduced so as to keep the total calories at 1800. It is questionable whether this additional carbohydrate produced any greater increase of glycosuria than would have resulted from the equivalent of fat.

After a fast day on Dec. 15, a low diet was begun and increased so as to show the tolerance without insulin, as discussed in this patient's history.

In January, on a diet of 60 gm. protein, 60 to 75 gm. carbohydrate, and 1800 to 2000 calories, the insulin dosage was increased as high as 44 units per day without entirely stopping glycosuria. Early in February, 48 units per day made the urine sugar-free. Then, beginning Feb. 12, the carbohydrate was increased to 100 gm., with a reduction of fat to keep the total calories at 2000. Glycosuria resulted from this change, and in

TABLE 7  
Case No. 989

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Aug. 19	50	80	20	1000	0	{ 129 178* }	35	..
20	"	"	"	"	0	{ 142 166* }	...	1
21	"	"	"	"	0	178*	...	2
22	60	115	30	1400	0	{ 92 217* }	...	2
23	"	"	"	"	0	{ 250 217* }	35	3
24	"	"	"	"	0	{ 258 200* }	...	3
25	"	"	"	"	++	{ 270 283* }	35	3
26	"	"	"	"	+	{ 263 132* }	...	5
27	"	"	"	"	0	{ 263 238* }	35	5
28	"	"	"	"	+	{ 270 220* }	...	5
29	"	"	"	"	0	{ 129 246* }	36	7
30	"	"	"	"	0	148	...	0
31	"	"	"	"	0	...	36	0
Sept. 1	"	"	"	"	++	312	...	7
2	"	"	"	"	+	200*	36	7
3	"	"	"	"	0	...	...	7
4	"	"	"	"	5.98	{ 306 334* }	36	7
5	60	15	40	535	5.32	306	...	7
6	"	"	"	"	1.89	366*	36	7
7	"	"	"	"	3.31	283	...	7
8	"	"	"	"	3.98	283*	36	7
9	"	"	"	"	4.80	250	...	7
10	"	"	"	"	4.80	...	36	7
11	"	"	"	"	0	{ 195 223* }	...	7
12	"	"	"	"	0	...	36	7
13	"	"	"	"	0	157	...	7
14-15	"	"	"	"	0	...	36	7
16	"	"	"	"	0	203	36	7
17-18	"	"	"	"	0	...	36	7
19	"	"	"	"	0	126	...	7
20	"	"	"	"	0	300*	36	7
21	"	"	"	"	0	139	...	6
22-23	"	"	"	"	0	...	...	6
24	"	"	"	"	0	{ 137 260* }	...	6
25-26	60	115	30	1400	0	...	36	6
27	"	"	"	"	0	270*	36	6
28	"	"	"	"	1.44	226	...	10
29	"	"	"	"	+	...	36	12
30	"	"	"	"	0	200*	...	14

TABLE 7.—Continued

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Oct.								
1-2	60	115	30	1400	+	...	35	16
3	"	"	"	"	0	...	35	18
4	"	"	"	"	0	164	...	18
5-7	"	"	"	"	0	...	37	18
8	"	"	"	"	0	254	...	18
9-11	60	93	30	1200	0	...	37	18
12	"	"	"	"	0	268*	...	18
13	"	"	"	"	0	156	...	18
14	"	"	"	"	0	272*	...	18
15	"	"	"	"	0	260	...	18
16-17	"	"	"	"	0	...	38	18
18	"	"	"	"	0	...	...	0
19	"	"	"	"	+	270	37	8
20-21	"	"	"	"	0	...	37	18
22	"	"	"	"	0	187	...	18
23-26	"	"	"	"	0	...	37	18
27	"	"	"	"	0	173	...	18
28-29	"	"	"	"	0	...	38	0
30	"	"	"	"	0	254	...	0
31	"	"	"	"	9 32	...	38	0
Nov.								
1	"	"	"	"	13.41	283	...	0
2	"	"	"	"	1.57	...	...	18
3	"	"	"	"	0	...	...	18
4	"	"	"	"	0	242	...	18
5-6	"	"	"	"	0	...	38	20
7	"	"	"	"	0	63	...	20
8	"	"	"	"	0	234*	...	20
9	"	"	"	"	0	209	38	20
10	"	"	"	"	0	105*	...	20
11	"	"	"	"	0	156	...	20
12	"	"	"	"	0	...	...	20
13-17	60	137	30	1600	0	...	39	20
18	"	"	"	"	0	211	40	20
19-21	"	"	"	"	0	...	40	20
22	"	"	"	"	0	...	40	20
23	"	"	"	"	0	234	...	20
24-27	"	"	"	"	0	...	40	20
28	"	"	"	"	0	168	41	20
29-30	"	"	"	"	0	...	...	20
Dec.								
1-2	"	"	"	"	+	...	...	20
3	60	160	30	1800	0	104	...	20
4-5	"	"	"	"	0	...	...	20
6	"	"	"	"	+	...	42	20
7-8	"	"	"	"	+	...	44	20
9	"	"	"	"	1 83	113*	...	20
10	60	146	60	1800	0	270	44	20
11	"	"	"	"	0	...	...	20
12	"	"	"	"	3 43	...	...	20
13	"	"	"	"	8.62	...	43	20
14	51	124	44	1191	8.58	...	...	20
15	Fasting				+	...	...	0
16	40	33	10	500	0	125	...	0
17	"	"	"	"	0	...	...	0



TABLE 7.—Continued

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Dec.	40	33	10	500				
18	"	"	"	"	0	132	42	0
19-20	50	62	10	800	0	...	41	0
21	"	"	"	"	0	192	...	0
22	"	"	"	"	0	...	41	0
23	50	85	10	1000	0	...	...	0
24-26	60	98	20	1200	0	...	...	4
27	60	122	40	1500	+	...	...	6
28	"	"	"	"	+	...	...	10
29	"	"	"	"	++	...	...	14
30-31	60	147	60	1800	+	...	...	20
1923								
Jan.								
1	"	"	"	"	++++	...	...	23
2	"	"	"	"	+++	...	...	26
3	"	"	"	"	2.98	...	...	26
4	"	"	"	"	...	...	44	26
5	"	"	"	"	33.54	...	...	26
6	"	"	"	"	10.21	...	45	26
7	"	"	"	"	16.57	...	...	28
8	"	"	"	"	15.96	...	46	28
9	"	"	"	"	...	...	...	30
10	"	"	"	"	14.01	...	...	32
11	"	"	"	"	11.95	...	46	32
12	"	"	"	"	23.33	...	...	32
13	"	"	"	"	9.72	...	46	36
14	"	"	"	"	5.82	...	...	36
15	"	"	"	"	5.84	...	48	36
16	"	"	"	"	11.01	...	...	36
17	"	"	"	"	1.26	...	...	40
18	"	"	"	"	0	...	48	40
19	"	"	"	"	0	...	...	40
20	60	162	75	2000	0	220	49	40
21	"	"	"	"	9.70	...	...	40
22	"	"	"	"	5.60	...	...	40
23	"	"	"	"	5.40	...	...	40
24	"	"	"	"	5.76	...	50	40
25	"	"	"	"	...	171	...	44
26	"	"	"	"	2.31	...	51	44
27	"	"	"	"	...	...	...	44
28	"	"	"	"	1.52	...	...	44
29	"	"	"	"	5.39	...	...	44
30	"	"	"	"	11.69	...	...	44
31	"	"	"	"	6.64	...	51	44
Feb.								
1	"	"	"	"	3.68	...	...	46
2	"	"	"	"	5.22	...	...	46
3	"	"	"	"	0	...	52	48
4	"	"	"	"	3.01	...	...	48
5	"	"	"	"	5.60	...	...	48
6-10	"	"	"	"	0	...	53	48
11	"	"	"	"	0	203	...	48
12-17	60	151	100	2000	0	...	54	48
18	"	"	"	"	0	226	54	48
19	"	"	"	"	0.64	...	...	48
20	"	"	"	"	0	...	...	48

TABLE 7.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.	60	151	100	2000				
21	"	"	"	"	7.70	...	56	50
22	"	"	"	"	...	...	...	50
23	"	"	"	"	2.22	...	57	50
24	"	"	"	"	13.10	...	57	52
25	"	"	"	"	17.09	...	...	52
26	"	"	"	"	15.66	...	56	52
27	"	"	"	"	22.68	...	...	54
28	"	"	"	"	12.74	...	56	54
Mar.								
1	"	"	"	"	15.45	...	...	56
2	"	"	"	"	2.52	...	56	56
3	"	"	"	"	+	...	...	60
4	"	"	"	"	11.45	...	57	60
5	"	"	"	"	0	...	...	62
6	"	"	"	"	+	...	57	62
7	"	"	"	"	+	...	...	64
8	"	"	"	"	+	...	...	66
9	"	"	"	"	++++	...	59	70
10	"	"	"	"	16.53	...	59	70
11	"	"	"	"	+	...	...	70
12	"	"	"	"	5.28	...	58	70
13	"	"	"	"	0	...	...	66
14	"	"	"	"	0	...	59	66
15	"	"	"	"	0	...	...	66
16	"	"	"	"	4.43	...	59	66
17	"	"	"	"	0	...	...	66
18	"	"	"	"	5.52	...	59	66†
19	"	"	"	"	3.89	187	...	66
20	"	"	"	"	+	157	60	66†
21	"	"	"	"	0	...	...	66
22	"	"	"	"	0	...	60	66
23	"	"	"	"	0	...	...	66
24	"	"	"	"	7.47	...	...	66†
25	"	"	"	"	0	101*	...	66†
26	"	"	"	"	15.20	142	60	66
27	"	"	"	"	0	93	...	66
28	"	"	"	"	0	171	...	66
29	"	"	"	"	54.80	...	...	16
30	"	"	"	"	38.00	...	58	52
31	80	120	150	2000	0	121	...	66
April								
1	"	"	"	"	65.40	...	...	66
2	"	"	"	"	17.70	...	...	66
3	"	"	"	"	28.60	...	...	66
4	"	"	"	"	22.90	...	...	66
5	"	"	"	"	0	...	61	66
6	"	"	"	"	40.80	...	...	70
7	"	"	"	"	0	...	...	70
8	"	"	"	"	7.00	...	...	70
9	"	"	"	"	0	...	61	70
10	"	"	"	"	0	...	...	70

\*Blood sample taken at 8 p.m.

†Other blood samples taken before breakfast.

‡Rest days.

TABLE 8  
Case No. 1069

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Nov.								
22	35	26	5	400	++++	484	58	4
23-24	"	"	"	"	++++	...	517	4
25	"	"	"	"	+++	416	...	4
26-27	"	"	"	"	++	...	58	4
28	"	"	"	"	++	416	57	2
29	"	"	"	"	+++	...	...	2
30	"	"	"	"	+++	...	57	4
Dec.								
1	"	"	"	"	+	...	58	4
2	"	"	"	"	++	357	...	4
3-4	35	60	5	700	++	...	57	4
5	"	"	"	"	0 69	...	...	4
6	"	"	"	"	6.72	455	...	2
7	"	"	"	"	8.57	...	57	4
8	"	"	"	"	8.27	...	57	8
9	"	"	"	"	+	441	...	8
10	"	"	"	"	16.93	...	57	0
11	"	"	"	"	6.30	...	...	4
12	"	"	"	"	10.04	...	57	8
13	"	"	"	"	3.72	441	...	8
14	"	"	"	"	3.11	...	...	10
15	"	"	"	"	3.93	...	56	10
16	"	"	"	"	3.25	366	56	10
17	"	"	"	"	4.46	...	...	10
18-19	"	"	"	"	+	...	57	10
20	"	"	"	"	+	366	...	10
21	"	"	"	"	+	...	57	10
22	"	"	"	"	+	...	...	10
23	"	"	"	"	+	385	57	10
24	35	13	5	280	0	...	...	10
25	35	20	5	340	0	...	57	10
26	35	16	5	300	0	270	58	10
27	35	19	5	330	0	...	...	10
28	35	25	5	385	0	...	58	10
29	35	10	5	250	0	...	...	10
30	"	"	"	"	0	242	58	10
31	"	"	"	"	0	...	...	10
1923								
Jan.								
1	"	"	"	"	0	223	58	10
2-3	"	"	"	"	0	...	...	10
4	"	"	"	"	0	184	57	10
5	"	"	"	"	0	...	...	10
6	"	"	"	"	0	169	58	10
7	35	10	20	310	0	...	...	10
8	35	20	20	400	0	156	59	10
9-10	35	13	20	340	0	...	...	10
11	35	10	20	310	0	...	57	10
12-13	"	"	"	"	0	150	57	10
14	35	53	20	700	0	171	59	10
15-16	"	"	"	"	0	...	59	10
17	"	"	"	"	0	211	59	10
18-19	"	"	"	"	0	...	59	10

TABLE S.—*Continued.*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
20	35	53	20	700	0	270	...	10
21	35	102	20	1138	0	...	59	10
22	"	"	"	"	0	214	...	10
23-24	35	120	20	1300	0	...	59	10
25	"	"	"	"	0	306	59	10
26	"	"	"	"	0	...	...	10
27	"	"	"	"	0	220	59	10
28	"	"	"	"	0	...	...	10
29	35	170	15	1730	0	...	59	10
30	"	"	"	"	0	192	...	10
31	"	"	"	"	0	...	60	10
Feb.								
1	"	"	"	"	0	195	...	10
2	"	"	"	"	0	...	61	10
3	"	"	"	"	0	220	...	10

the subsequent experience up to March 31, the insulin dosage required to prevent glycosuria on this diet was found to be between 66 and 70 units per day. This increase of approximately 22 units of insulin seems altogether too great to correspond to the increase of only 25 gm. of carbohydrate, and it is therefore probable that the rapidly increasing body weight was one important factor in the increase of the insulin requirement.

The patient ordinarily took considerable exercise by walking and in gymnasium. Trials of nearly complete rest were made on March 18, 20, 24 and 25. The results were not decisive, perhaps because the actual differences between days of exercise and rest were not sufficiently great. The urine was sugar-free on the rest day of March 25, but an after-effect of the rest seemed to be evident in the unusually heavy glycosuria of March 26. This glycosuria cleared up with continued exercise on March 27 and 28. Also, the three days, March 21-23, were spent at home, where playing with the other children involved much more vigorous exercise than had been taken at the Institute, and on these days the urine was completely sugar-free. On the whole, therefore, there seemed to be a tendency of exercise to prevent glycosuria.\*

Beginning March 31, the carbohydrate was increased to 150 gm. while fat was reduced to keep the total calories at 2000. Glycosuria resulted from this change. The sugar-free days of April 5 and 7 alternating with days of heavy glycosuria are peculiar, but violations of diet and losses of urine were excluded, and the explanation is probably to be found in variations in the amount of exercise taken. It became necessary to increase the insulin to 70 units per day. The patient was discharged on this

\* Since the return home, the nurse reports that glycosuria can be made to come or go according to the amount of exercise the child takes on different days.

program April 10, and has continued to have no glycosuria or only occasional traces. The amount of insulin which seemed to balance the increase of carbohydrate in this instance was small, and doubt is again cast upon the feasibility of attempts to reckon the insulin requirement according to the carbohydrate of the diet.

#### REMARKS ON TABLE 8.

With the gradual increase of diet and dosage, it was found here that 10 units of insulin per day did not quite suffice to abolish glycosuria on the diet of 35 gm. protein, 5 gm. carbohydrate and 700 calories up to Dec. 23. Withdrawal of most of the fat, beginning Dec. 24, immediately stopped glycosuria and reduced the blood sugar below 0.2 per cent. by Jan. 5 and 6. The glucose value of the diet was not the explanation, for increase of the carbohydrate to 20 gm. daily for the week Jan. 7-13 did not prevent a further fall of the plasma sugar to 0.150 per cent. on the latter date. Beginning Jan. 14, fat was introduced so as to raise the total calories again to 700 daily for the following week. Accordingly, the plasma sugar rose steadily to 0.270 per cent. on Jan. 20.

Further increases were then made, resulting in a ration of 35 gm. protein, 15 gm. carbohydrate and 1730 calories per day. There was no glycosuria, and the plasma sugar was actually somewhat lower from Jan. 30 to Feb. 3 than on Jan. 20. This result is by no means exceptional, but is merely an illustration of the deceptive delay of the signs of injury from fat in many cases. We are sufficiently assured from other tests of this kind that glycosuria would have resulted from the increase of fat and calories if sufficient time had been given. The experiment had to be discontinued at this point in order to build up a balanced diet preparatory to discharge.

The tests first described show that a Newburgh diet of 700 calories is less effective in controlling glycosuria and hyperglycemia than a lower fat allowance making up only about 300 calories per day.

#### REMARKS ON TABLE 9.

With the diet of 20 gm. protein, 1 gm. carbohydrate and 350 calories up to Nov. 15, 2 units of insulin daily was probably an overdose, for it reduced the plasma sugar rapidly and hypoglycemic symptoms would probably have occurred soon.

Beginning Nov. 16, 101 gm. of fat was added, so as to raise the diet to 20 gm. protein, 1 gm. carbohydrate and 1254 calories. The plasma sugar rose steadily, and notwithstanding an increase of insulin to 4 units, traces of glycosuria were present from Nov. 25 to 30, ceasing then when the insulin was raised to 6 units. A further increase to 8 units did not succeed in reducing the plasma sugar to normal on this diet from Dec. 8 to 28.

Dec. 29 to Jan. 7, the protein and carbohydrate were increased to 40 gm. each and fat reduced so as to keep the total calories constant at 1254. Glycosuria began Jan. 2, and notwithstanding an increase of insulin to 12 units on the following day, it increased to 32.17 gm. by Jan. 7.

TABLE 9  
Case No. 1065

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Nov.									
12	20	29	1	350	0	.....	234	78	1
13	"	"	"	"	0	.....	.....	..	2
14	"	"	"	"	0	.....	129	..	2
15	"	"	"	"	0	.....	097	74	2
16	20	130	1	1254	0	.....	072	..	2
17	"	"	"	"	0	.....	171	75	2
18	"	"	"	"	0	.....	..	..	2
19	"	"	"	"	0	.....	..	..	2
20	"	"	"	"	0	.....	214	74	2
21	"	"	"	"	0	.....	..	..	2
22	"	"	"	"	0	.....	272	71	2
23	"	"	"	"	0	.....	..	..	4
24	"	"	"	"	0	.....	..	..	4
25	"	"	"	"	+	.....	385	71	4
26	"	"	"	"	+	.....	..	..	4
27	"	"	"	"	+	.....	366	..	4
28	"	"	"	"	+	.....	334	71	4
29	"	"	"	"	+	.....	..	..	4
30	"	"	"	"	+	.....	..	..	4
Dec.									
1	"	"	"	"	0	.....	..	..	6
2	"	"	"	"	0	.....	272	..	6
3	"	"	"	"	0	.....	..	..	6
4	"	"	"	"	0	.....	..	73	6
5	"	"	"	"	0	.....	258	..	6
6	"	"	"	"	0	.....	..	73	3
7	"	"	"	"	0	.....	211	..	6
8	"	"	"	"	0	.....	..	72	8
9	"	"	"	"	0	.....	..	..	8
10	"	"	"	"	0	.....	159	72	8
11	"	"	"	"	0	.....	..	..	8
12	"	"	"	"	0	.....	206	72	8
13	"	"	"	"	0	.....	..	..	8
14	"	"	"	"	0	.....	..	71	8
15	"	"	"	"	0	.....	180	..	8
16	"	"	"	"	0	.....	..	73	8
17	"	"	"	"	0	.....	217	..	8
18	"	"	"	"	0	.....	..	72	8
19	"	"	"	"	0	.....	..	..	8
20	"	"	"	"	0	.....	..	72	8
21	"	"	"	"	0	.....	..	..	8
22	"	"	"	"	0	.....	..	73	8
23	"	"	"	"	0	.....	..	..	8
24	"	"	"	"	0	.....	164	74	8
25	"	"	"	"	0	.....	..	..	8
26	"	"	"	"	0	.....	200	74	8
27	"	"	"	"	0	.....	211	..	8
28	"	"	"	"	0	.....	..	68	8
29	40	103	40	1254	0	.....	..	..	8
30	"	"	"	"	0	.....	..	72	8
31	"	"	"	"	0	.....	..	..	8
1923									
Jan.									
1	"	"	"	"	0	.....	..	73	8
2	"	"	"	"	+	.....	341	..	8



TABLE 9.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan. 3	40	103	40	1254	+++	.....	...	72	12
4	"	"	"	"	+++++	.....	...	72	12
5	"	"	"	"	+++++	.....	357	73	12
6	"	"	"	"	+++++	.....	...	73	12
7	"	"	"	"	32.17	9.20	...	73	12
8	50	126	40	1500	19.58	7.21	416	73	12
9	"	"	"	"	10.16	4.18	...	73	12
10	"	"	"	"	12.87	6.95	...	73	15
11	"	"	"	"	++	10.04	429	73	15
12	"	"	"	"	++	8.25	...	73	15
13	"	"	"	"	9.25	8.39	...	75	15
14	"	"	"	"	6.49	6.76	...	75	18
15	"	"	"	"	+	9.99	326	75	18
16	"	"	"	"	+	5.79	...	75	18
17	"	"	"	"	+	12.54	...	75	18
18	"	"	"	"	+	8.30	366	75	18
19	"	"	"	"	+	7.34	...	75	22
20	"	"	"	"	0	9.27	306	74	22
21	"	"	"	"	0	5.25	226	74	22
22	"	"	"	"	0	8.83	319	74	30
23	"	"	"	"	0	7.16	...	74	30
24	"	"	"	"	0	5.79	258	75	30
25	"	"	"	"	0	....	171	75	30

According to the evidence of other experiments, the protein increase can be practically excluded from consideration and the glycosuria can be attributed essentially to the increase of carbohydrate. As the patient had to be prepared for discharge, it was impossible to take time to learn the exact insulin requirement for balancing this increase. Probably the 12 units would nearly have sufficed, as judged by the rapid reduction of glycosuria, and by the results from 15 and 18 units with the subsequent higher diet.

From Jan. 8 to discharge on Jan. 25 the diet was 50 gm. protein, 40 gm. carbohydrate and 1500 calories. Apparently this was for the first time a *luxus* diet, for the body weight began to increase, and the glycosuria and marked hyperglycemia were not controlled until the insulin had been increased to 30 units daily.

This experiment shows a stronger glycosuric influence of carbohydrate as compared with the same number of calories of fat, but the dominant status of the total calories of the diet is still evident.

#### REMARKS ON TABLE 10.

This patient's record is given in full in order to show two things.

The first is the great severity of the case, indicated by (a) the long time required (Aug. 19 to Sept. 3) to abolish glycosuria on the very low diet; (b) the unusual hyperglycemia persisting on the semi-starvation

TABLE 10  
Case No. 1212

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Aug.								
19	20	6	0	134	....	...	...	..
20	20	7	0	143	+++	...	78	..
21	20	6	0	134	+++	429	...	..
22	20	2	0	98	+++	...	79	..
23-24	20	6	0	134	++	...	82	..
25	20	7	0	143	+++	375	...	..
26-28	20	7	0	143	+	...	85	..
29	20	2	0	98	+	349	...	..
30-31	20	4	0	116	+	...	87	..
Sept.								
1	Fast				+	...	87	..
2	20	2	0	98	+	...	...	..
3	Fast				+	...	90	..
4	20	5	0	125	0	...	...	..
5-6	Fast				0	...	91	..
7	20	1	0	89	0	326	91	..
8	Fast				0	...	...	..
9	20	0	0	80	0	...	91	..
10	Fast				0	...	...	..
11	20	1	0	89	0	270	91	..
12	Fast				0	306	...	..
13	20	4	0	116	0	...	...	..
14	Fast				0	...	...	..
15	20	4	0	116	0	272	91	..
16	Fast				0	...	...	..
17	5	9	0	101	0	...	...	..
18	"	"	"	"	0	341	...	..
19-20	"	"	"	"	0	...	91	..
21	"	"	"	"	0	366	95	..
22	"	"	"	"	0	{ 334 263* }	...	1
23	"	"	"	"	0	288*	86	1
24	"	"	"	"	0	334	...	1
25	"	"	"	"	0	...	84	1
26	"	"	"	"	0	275	...	1
27	"	"	"	"	0	...	84	1
28	"	"	"	"	0	...	...	1
29	"	"	"	"	0	{ 180 182* }	85	1
30	"	"	"	"	0	...	...	1
Oct.								
1	"	"	"	"	0	211	...	1
2	"	"	"	"	0	199	...	1
3	25	0	0	100	0	200	84	1
4	"	"	"	"	0	...	...	1
5	25	2	0	118	0	...	83	1
6	25	5	0	145	0	{ 217 200* }	...	1
7	"	"	"	"	0	...	83	1
8	25	43	2	500	0	...	...	1
9	"	"	"	"	0	...	...	1
10	"	"	"	"	0	226	...	1
11	"	"	"	"	0	254	83	1

\*Blood sample taken at 8 p. m.

Other blood samples taken before breakfast.

TABLE 10.—*Continued*

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Oct.								
12	25	43	2	500	0	230*	...	1
13	"	"	"	"	0	282	85	1
14	"	"	"	"	0	288*	...	1
15	"	"	"	"	0	230	85	1
16-17	"	"	"	"	0	...	83	1
18	"	"	"	"	0	...	...	0
19	"	"	"	"	0	226	81	1½
20-21	"	"	"	"	0	...	82	1
22	"	"	"	"	0	214	...	1
23-25	"	"	"	"	0	...	81	2
26	"	"	"	"	0	182	...	2
27-28	"	"	"	"	0	...	82	2
29	30	62	5	700	0	162	81	2
30-31	"	"	"	"	0	...	80	2
Nov.								
1	"	"	"	"	0	...	...	2
2	40	66	10	800	0	173	82	2
3-4	40	88	10	1000	0	...	83	2
5	50	104	15	1200	0	242	...	3
6	"	"	"	"	0	...	84	3
7-8	"	"	"	"	+	...	85	6
9	"	"	"	"	0	242	...	6
10	"	"	"	"	+	...	85	6
11	"	"	"	"	0	272	...	6
12	"	"	"	"	++++	...	...	8
13	"	"	"	"	+	...	...	9
14	"	"	"	"	+++	...	83	9
15	"	"	"	"	+++	272	...	9
16-17	"	"	"	"	++++	...	82	9
18	"	"	"	"	++++	319	...	9
19-21	"	"	"	"	++++	...	83	9
22	"	"	"	"	++++	...	...	6
23	"	"	"	"	++++	366	83	12
24-26	"	"	"	"	++++	...	81	12
27	"	"	"	"	+	283	...	12
28	"	"	"	"	0	...	81	6
29	"	"	"	"	+++	334	...	6
30	"	"	"	"	+++	...	81	12
Dec.								
1	"	"	"	"	+	...	...	12
2	"	"	"	"	0	254	...	12
3-4	"	"	"	"	0	...	81	12
5	"	"	"	"	14 69	...	...	9
6	"	"	"	"	++	...	85	9
7	"	"	"	"	0	294	...	12
8	"	"	"	"	0	...	85	16
9	"	"	"	"	10 39	...	...	16
10	"	"	"	"	++++	...	86	12
11	"	"	"	"	7 08	...	...	12
12	"	"	"	"	31 07	326	87	16
13	"	"	"	"	36 75	405	...	16
14	"	"	"	"	20 19	...	91	20
15	"	"	"	"	22 26	...	...	20
16	"	"	"	"	++++	385	86	20

\*Blood sample taken at 8 p.m.

Other blood samples taken before breakfast.

program to Sept. 21; (c) the continuance of hyperglycemia with minimal diets and 1 unit of insulin daily to Oct. 7; (d) the heavy glycosuria with 20 units of insulin daily on the final 1200 calory diet.

The second is the increased functional burden placed upon the pancreatic islands by even a moderate diet. This patient became free from glycosuria without insulin, and remained so on a regime approximating starvation. In October she tolerated 25 gm. protein, 2 gm. carbohydrate and 500 calories with only 1 unit of insulin daily, and with 2 units the fall of plasma sugar from Oct. 23 to 29 indicated that normal or sub-normal plasma sugar levels would soon have been reached. The diet was then gradually increased to 50 gm. protein, 15 gm. carbohydrate and 1200 calories, and doses of 12, 16 and 20 units of insulin then proved inadequate to control the glycosuria. The increase of protein and carbohydrate could not possibly explain this increased insulin requirement, for the following reasons: (a) all authors are agreed that these quantities of insulin should provide for the metabolism of more glucose than was represented in this increase; (b) at the height of the glycosuria (Dec. 12-15) practically none of the glucose represented in this increase was metabolized, for the urinary glucose was almost equal to the theoretical difference in glucose between the 500 calory and the 1200 calory diets; (c) our numerous tests with other patients furnish sufficient evidence that if the fat had been omitted, the insulin dosage mentioned would have provided for the metabolism of the 50 gm. protein and 15 gm. carbohydrate, probably to the extent of causing hypoglycemia. The tremendous increase of insulin requirement must therefore be ascribed chiefly to the fat. The basal metabolism of this patient during semi-starvation was probably well below 1200 calories; but as she was never bedfast, it is doubtful if her actual daily expenditure of energy was much below this figure or if she could have been kept alive on a much lower diet. Broadly speaking, then, the difference between a semi-starvation regime and a maintenance diet may be something like 20 units of insulin per day.

An idea may thus be formed of the difference in the demands imposed upon the pancreatic islands by a fasting or rigid undernutrition treatment and by the high fat diets of Newburgh and Marsh. The error of those who attempt to calculate the insulin requirement of patients merely on the glucose of the diet or of the urine is equally evident.

#### REMARKS ON TABLE 11.

The high D/N ratios with the very low diets up to Jan. 1, 1922, were regarded as evidence that the condition was impossible of control. As mentioned in paper 1, these ratios were evidently somewhat elevated by carbohydrate derived from the "fillers" in the diet.

The length of the preliminary observations is sufficient to prove that the condition was not subject to spontaneous change. The results obtained with insulin treatment beginning Aug. 10 are therefore decisive. Dosage of 4 to 8 units per day seemed necessary to keep the urine sugar-

TABLE 11  
Case No. 1034

Date	Diet				URINE				Blood		Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C. H. Gm.	Cal.	Total Acetone Gm.	NH <sub>3</sub> Gm.	Nitrogen Gm.	Sugar Gm.	D:N Ratio	Plasma Sugar Mg. per 100 cc.	CO <sub>2</sub> Vol. %	
1921												
Nov.												
19	15	0	0	60	....	....	....	++	....	....	....	....
20-21	"	"	"	"	....	....	....	++	....	....	....	....
22	15	3	0	87	....	....	....	++	....	625	....	....
23	"	"	"	"	....	....	....	++	....	....	....	....
24	20	18	10	282	....	....	....	++	....	....	....	....
25-27	15	1	3	81	....	....	....	++	....	600	....	....
28	"	"	"	"	....	....	4.30	++	....	....	....	....
29	"	"	"	"	....	....	6.18	++	....	....	....	....
30	"	"	"	"	....	....	....	14.31	....	....	....	....
Dec.								22.17	....	....	....	....
1	"	"	"	"	....	....	....	12.37	....	....	....	....
2	Fast	1	3	81	....	....	....	19.69	....	....	....	....
3	Fast	0	0	40	....	....	....	++	....	....	....	....
4	Fast	0	0	40	....	....	....	++	....	....	....	....
5	Fast	0	0	40	....	....	....	++	....	....	....	....
6	Fast	0	0	40	....	....	....	++	....	....	....	....
7	Fast	0	0	40	....	....	....	++	....	....	....	....
8	Fast	0	0	40	....	....	....	++	....	500	....	....
9	Fast	0	0	40	....	....	....	++	....	....	....	....
10	Fast	0	0	40	....	....	....	++	....	....	....	....
11	Fast	0	0	40	....	....	....	++	....	....	....	....
12	Fast	0	0	40	....	....	....	++	....	536	....	....
13	Fast	0	0	40	....	....	....	++	....	....	....	....
14	"	"	"	"	....	....	2.11	4.70	....	....	....	....
15	"	"	"	"	....	....	5.31	9.42	....	....	....	....
					....	....	....	22.75	4.46	....	....	....
					....	....	....	....	4.28	....	....	....

TABLE 11.—Continued

16	50	20	0	380	.....	.....	10 77	6 80	.....	4 91	.....	750	.....	40	.....
17	"	"	"	"	.....	.....	4 52	52 97	.....	4 96	.....	750	.....	40	.....
18	"	"	"	"	.....	.....	8 87	22 42	.....	.....	50 4	.....	.....	40	.....
19	"	"	"	"	.....	.....	7 51	35 70	.....	4 75	.....	.....	.....	40	.....
20	"	"	"	"	.....	.....	1 17	61 53	.....	5 31	.....	.....	.....	40	.....
21	"	"	"	"	.....	.....	1 75	50 26	.....	4 59	.....	.....	.....	40	.....
22	"	"	"	"	.....	.....	1 61	49 52	.....	3 98	.....	.....	.....	40	.....
23	"	"	"	"	.....	.....	2 01	.....	.....	.....	.....	.....	.....	41	.....
24	"	"	"	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	41	.....
25	35	45	0	550	.....	.....	.....	.....	.....	.....	.....	.....	.....	42	.....
26	50	16	0	344	.....	.....	3 12	40 87	.....	5 53	.....	.....	.....	42	.....
27	"	"	"	"	.....	.....	2 68	31 17	.....	4 45	.....	.....	.....	44	.....
28	"	"	"	"	.....	.....	1 39	31 28	.....	4 17	.....	.....	.....	44	.....
29	"	"	"	"	.....	.....	0 88	31 36	.....	3 65	.....	.....	.....	44	.....
30	"	"	"	"	.....	.....	0 93	.....	.....	.....	.....	.....	.....	44	.....
31	"	"	"	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	44	.....
1922	"	"	"	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	44	.....
Jan.	"	"	"	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	44	.....
1	"	"	"	"	.....	.....	0 47	21 79	.....	3 71	.....	.....	.....	45	.....
2	50	50	0	650	.....	.....	1 25	35 16	.....	3 38	.....	.....	.....	45	.....
3	"	"	"	"	.....	.....	.....	21 59	.....	3 53	.....	.....	.....	45	.....
4	"	"	"	"	.....	.....	1 45	32 33	.....	3 62	.....	.....	.....	44	.....
Feb.	"	"	"	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	44	.....
6-12	60	60	6	801	.....	.....	.....	.....	.....	.....	.....	.....	.....	36	.....
13	"	"	"	"	.....	.....	10 79	31 49	.....	2 36	.....	.....	.....	36	.....
14	"	"	"	"	.....	.....	5 90	17 17	.....	1 89	.....	600	.....	35	.....
15	"	"	"	"	.....	.....	13 54	37 01	.....	2 29	.....	.....	.....	35	.....
16	"	"	"	"	.....	.....	7 73	26 50	.....	2 65	.....	.....	.....	33	.....
17	"	"	"	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	33	.....
18	"	"	"	"	.....	.....	8 44	18 45	.....	1 47	.....	.....	.....	36	.....
19	"	"	"	"	.....	.....	10 50	33 75	.....	2 61	.....	.....	.....	36	.....
20	"	"	"	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	36	.....
21	"	"	"	"	.....	.....	8 66	21 35	.....	1 77	.....	.....	.....	36	.....
22	"	"	"	"	.....	.....	7 64	21 31	.....	2 00	.....	.....	.....	34	.....
23	"	"	"	"	.....	.....	10 67	39 53	.....	3 14	.....	.....	.....	34	.....
24	"	"	"	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	37	.....
25	"	"	"	"	.....	.....	1 88	20 75	.....	2 50	.....	.....	.....	37	.....
26	"	"	"	"	.....	.....	3 01	21 80	.....	2 34	.....	682	.....	37	.....
27	"	"	"	"	.....	.....	4 07	34 94	.....	3 33	.....	.....	.....	37	.....
	"	"	"	"	.....	.....	6 89	18 16	.....	1 76	.....	.....	.....	37	.....



TABLE 11.—Continued

Date 1922	DIET				URINE					BLOOD		Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C. H. Gm.	Cal.	Total Acetone Gm.	NH <sub>3</sub> Gm.	Nitrogen Gm.	Sugar Gm.	D.N. Ratio	Plasma Sugar Mg. per 100 cc.	CO <sub>2</sub> Vol. %		
Feb. 28	60	60	6	804	....	2.42	9.02	26.48	2.27	....	....	....	....
Mar. 1	"	"	"	"	6.52	3.64	9.70	27.53	2.22	....	....	....	....
2	"	"	"	"	8.07	3.64	9.17	41.31	3.85	....	....	....	....
3	"	"	"	"	9.24	2.49	10.97	34.42	2.59	....	....	....	....
4	"	"	"	"	19.34	4.45	8.81	41.05	3.97	....	....	....	....
5	"	"	"	"	....	4.27	8.25	39.67	4.08	....	....	....	....
6	"	"	"	"	....	3.95	9.99	40.35	3.43	....	....	30	....
7	"	"	"	"	....	3.92	10.23	29.95	2.34	....	....	....	....
8	"	"	"	"	....	4.50	9.61	38.43	3.37	....	....	32	....
9	"	"	"	"	....	6.41	9.00	37.00	3.44	....	....	....	....
10	"	"	"	"	....	4.92	10.01	48.55	4.25	....	....	....	....
11	"	"	"	"	....	4.74	8.13	32.89	3.30	....	51.9	30	....
28	"	"	"	"	....	2.38	10.61	42.90	3.48	833	....	30	....
Apr. 10	"	"	"	"	....	....	9.77	44.70	3.96	714	....	31	....
29	"	"	"	"	....	....	9.80	36.70	3.13	600	....	....	....
May 16	"	"	"	"	....	....	10.20	38.10	3.83	416	....	31	....
June 25	"	"	"	"	0.25	....	10.00	35.20	2.92	555	....	25	....
26	"	"	"	"	0.17	....	7.40	31.00	3.37	....	....	....	....
27	"	"	"	"	4.89	....	6.40	21.60	2.43	....	....	25	....
28	"	"	"	"	9.58	....	9.30	36.10	3.23	....	....	....	....
29	"	"	"	"	5.14	....	8.50	33.90	3.28	....	....	30	....
30	"	"	"	"	1.04	....	9.6	38.30	3.36	....	....	....	....

TABLE 11.—Continued

July	60	60	801	1.81	.....	9.7	33.60	2.84	.....	.....	28
1	60	60	801	2.01	.....	8.4	34.50	3.39	.....	.....	28
2	60	60	801	6.47	.....	8.2	31.60	3.12	.....	.....	28
3	60	60	801	4.11	.....	6.9	30.50	3.55	.....	.....	30
4	60	60	801	1.02	.....	10.1	28.00	2.16	.....	.....	30
5	60	60	801	7.21	.....	7.7	26.00	2.59	.....	.....	30
6	60	60	801	11.69	.....	8.3	27.00	2.53	.....	.....	30
7	60	60	801	2.71	.....	8.7	36.90	3.55	.....	.....	32
8	60	60	801	4.23	.....	10.9	46.00	3.66	.....	.....	32
9	60	60	801	4.00	.....	9.9	43.60	3.69	.....	.....	30
10	60	60	801	1.56	.....	10.8	37.10	3.43	.....	.....	30
11	60	60	801	2.29	.....	8.5	26.60	2.42	.....	.....	30
12	60	60	801	4.08	.....	9.7	40.80	3.58	.....	.....	30
13	60	60	801	3.04	.....	8.7	32.10	3.00	.....	.....	30
14	60	60	801	2.57	.....	9.6	20.40	1.50	.....	.....	30
15	60	60	801	1.78	.....	8.9	31.50	2.86	.....	.....	31
16	60	60	801	5.96	.....	7.5	30.80	3.30	.....	.....	31
17	60	60	801	2.47	.....	9.8	37.00	3.16	.....	.....	30
18	60	60	801	3.48	.....	7.7	30.00	3.11	.....	.....	30
19	60	60	801	2.65	.....	9.2	36.00	3.26	.....	.....	32
20	60	60	801	4.61	1.76	8.6	36.00	3.48	.....	.....	32
21	60	60	801	2.71	2.00	10.6	37.00	2.92	.....	.....	33
22	60	60	801	4.83	2.60	11.0	33.80	2.52	.....	.....	33
23	60	60	801	6.24	2.39	9.4	33.90	2.96	.....	.....	33
24	60	60	801	5.16	2.37	8.7	22.90	1.94	.....	.....	33
25	60	60	801	4.06	2.62	10.8	42.00	3.33	.....	.....	33
26	60	60	801	4.73	2.99	9.8	42.00	3.67	.....	.....	33
27	60	60	801	3.22	3.49	8.4	32.40	3.14	.....	.....	33
28	60	60	801	3.22	1.35	11.8	46.20	3.40	.....	.....	33
29	60	60	801	1.90	1.90	9.7	40.80	3.58	.....	.....	33
30	60	60	801	2.82	4.19	8.3	35.00	3.49	.....	.....	33
31	60	60	801	9.83	1.81	8.1	35.80	3.67	.....	.....	34
Aug.	60	60	801	2.66	3.28	8.7	28.80	2.62	.....	.....	34
1	60	60	801	3.13	4.86	7.9	27.60	2.73	.....	.....	35
2	60	60	801	5.31	3.28	8.6	38.40	3.76	.....	.....	35
3	60	60	801	7.47	2.06	9.5	26.70	2.17	.....	.....	35

TABLE 11.—Continued

Date 1922	DIET				URINE				BLOOD		Weight, Lb.	Insulin, Units	
	P. Gm.	F. Gm.	C. H. Gm.	Cal.	Total Acetone Gm.	NH <sub>3</sub> Gm.	Nitrogen Gm.	Sugar Gm.	D.N. Ratio	Plasma Sugar Mg per 100 cc.			CO <sub>2</sub> Vol. %
Aug.	60	60	6	804	1.70	2.54	8.9	34.50	3.20	.....	.....	32	.....
8	"	"	"	"	8.30	2.53	7.4	29.00	3.10	.....	.....	33	2 $\frac{3}{4}$
9	"	"	"	"	2.08	1.64	6.0	70.40	10.73	577	.....	33	1 $\frac{1}{2}$
10	"	"	"	"	.....	2.61	6.3	55.80	7.90	577	.....	32	5 $\frac{1}{2}$
11	"	"	"	"	.....	2.83	4.5	6.48	.....	518	.....	8	8
12	"	"	"	"	.....	2.30	7.2	7.22	.....	385	.....	8	8
13	"	"	"	"	.....	3.06	6.6	0	.....	220	.....	7	7
14	"	"	"	"	.....	.....	.....	0	.....	91	.....	4	4
15	"	"	"	"	.....	2.08	5.6	0	.....	180	.....	31	3 $\frac{1}{2}$
16	"	"	"	"	.....	.....	.....	0	.....	.....	.....	4	4
17	"	"	"	"	.....	.....	.....	0	.....	166	.....	.....	4
18	"	"	"	"	.....	.....	.....	0	.....	.....	.....	33	4
19	"	"	"	"	.....	.....	.....	0	.....	173	.....	4	4
20-24	"	"	"	"	.....	.....	.....	0	.....	.....	.....	4	4
25	"	"	"	"	.....	.....	.....	0	.....	.....	.....	4	4
26-29	"	"	"	"	.....	.....	.....	0	.....	242	.....	33	2
30	"	"	"	"	.....	.....	.....	++	.....	334	.....	33	0
31	"	"	"	"	.....	.....	.....	++	.....	.....	.....	.....	0
Sept.													
1	"	"	"	"	.....	.....	.....	+	.....	405	.....	34	4
2	"	"	"	"	.....	.....	.....	0	.....	263	.....	.....	4
3	"	"	"	"	.....	.....	.....	+	.....	304	.....	35	4
4	"	"	"	"	.....	.....	.....	0	.....	.....	.....	.....	4
5-7	"	"	"	"	.....	.....	.....	+	.....	.....	.....	34	4
8-9	"	"	"	"	.....	.....	.....	+	.....	.....	.....	36	6
10	"	"	"	"	.....	.....	.....	+	.....	.....	.....	8	8
11-12	"	"	"	"	.....	.....	.....	+	.....	.....	.....	8	8
13	"	"	"	"	.....	.....	.....	0	.....	375	.....	36	8
14-20	60	10	6	354	.....	.....	.....	+	.....	.....	.....	35	8

TABLE 11.—Continued

21	60	10	6	354	.....	.....	0	.....	214	.....	35	4
22-23	"	"	"	"	.....	.....	0	.....	.....	.....	35	4
24	"	"	"	"	.....	.....	0	.....	168	.....	.....	4
25-27	"	"	"	"	.....	.....	0	.....	.....	.....	35	4
28-30	"	"	"	"	.....	.....	0	.....	.....	.....	35	8
Oct.												
1	"	"	"	"	.....	.....	0	.....	.....	.....	35	8
2	"	"	"	"	.....	.....	0	.....	137	.....	.....	8
3	60	10	11	374	.....	.....	0	.....	187	.....	35	8
4	"	"	"	"	.....	.....	0	.....	.....	.....	.....	8
5	"	"	"	"	.....	.....	0	.....	123	.....	35	8
6	"	"	"	"	.....	.....	0	.....	115	.....	.....	6
7-9	"	"	"	"	.....	.....	0	.....	.....	.....	35	6
10	"	"	"	"	.....	.....	0	.....	223	.....	.....	6
11-12	"	"	"	"	.....	.....	0	.....	.....	.....	35	6
13	"	"	"	"	.....	.....	0	.....	230	.....	35	6
14	"	"	"	"	.....	.....	0	.....	.....	.....	.....	6
15	"	"	"	"	.....	.....	0	.....	263	.....	35	6
16	"	"	"	"	.....	.....	0	.....	.....	.....	.....	6
17	"	"	"	"	.....	.....	0	.....	.....	.....	35	6
18	"	"	"	"	.....	.....	0	.....	.....	.....	.....	0
19	"	"	"	"	.....	.....	0	.....	.....	.....	35	3
20	"	"	"	"	.....	.....	+	.....	455	.....	.....	6
21	"	"	"	"	.....	.....	+	.....	.....	.....	36	6
22	"	"	"	"	.....	.....	0	.....	.....	.....	.....	6
23	"	"	"	"	.....	.....	0	.....	203	.....	36	6
24	"	"	"	"	.....	.....	0	.....	.....	.....	.....	6
25	"	"	"	"	.....	.....	0	.....	.....	.....	36	6
26	"	"	"	"	.....	.....	0	.....	.....	.....	.....	6
27	"	"	"	"	.....	.....	0	.....	178	.....	36	6
28	"	"	"	"	.....	.....	0	.....	.....	.....	.....	6
29-30	"	"	"	"	.....	.....	0	.....	.....	.....	36	8
31	"	"	"	"	.....	.....	0	.....	91	.....	36	8
Nov.												
1-2	60	60	6	804	.....	.....	0	.....	.....	.....	36	8
3	"	"	"	"	.....	.....	0	.....	98	.....	.....	8
4-6	"	"	"	"	.....	.....	0	.....	.....	.....	36	8
7	"	"	"	"	.....	.....	0	.....	143	.....	36	8

TABLE 11.—Continued

Date 1922	DIET				URINE				BLOOD		Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C. H. Gm.	Cal.	Total Acetone Gm.	NH <sub>3</sub> Gm.	Nitrogen Gm.	Sugar Gm.	D-N Ratio	Plasma Sugar Mg. per 100 cc.	CO <sub>2</sub> Vol. %	
Nov. 8-9	60	"	6	804	....	....	....	0	....	....	....	8
10	"	"	"	"	....	....	....	0	....	166	....	8
11-13	"	"	"	"	....	....	....	0	....	....	....	8
14†	"	"	"	"	....	....	....	0	....	178	....	8
Dec. 26	"	"	"	"	....	....	....	++	....	....	....	8
27	"	"	"	"	....	....	....	10.82	....	455	....	8
28	"	"	"	"	....	....	6.50	+	....	....	....	8
29	"	"	"	"	....	....	4.97	+	....	405	....	8
30	"	"	"	"	....	....	6.35	+	....	....	....	8
31	"	"	"	"	....	....	5.99	+	....	....	....	8
1923 Jan. 1	"	"	"	"	....	....	7.94	+	....	....	....	8
2	"	"	"	"	....	....	6.40	+	....	....	....	8
3	"	"	"	"	....	....	10.03	+	....	441	....	8
4	"	"	"	"	....	....	11.36	+	....	....	....	8
5	"	"	"	"	....	....	4.82	+	....	....	....	8
6	"	"	"	"	....	....	4.42	+	....	....	....	8
7	"	"	"	"	....	....	5.48	+	....	....	....	8
8	"	"	"	"	....	....	....	+	....	....	....	8
9	"	"	"	"	....	....	....	0	....	....	....	8
10	"	"	"	"	....	....	7.88	0	....	....	....	8
11	"	"	"	"	....	....	5.86	0	....	312	....	8
12	"	"	"	"	....	....	....	0	....	....	....	8
13	"	"	"	"	....	....	5.08	0	....	....	....	8
14	"	"	"	"	....	....	5.46	0	....	....	....	8

TABLE 11.—Continued

[illegible]



TABLE II.—Continued

Date 1923	DIET				URINE				Blood		Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C. H. Gm.	Cal.	Total Acetone Gm.	NH <sub>3</sub> Gm.	Nitrogen Gm.	Sugar Gm.	D:N Ratio	Plasma Sugar Mg. per 100 cc.	CO <sub>2</sub> Vol. %	
Feb.												
21	Fast				....	....	4.88	13.76	....	429	....	0
22	Fast				....	....	2.44	+	....	349	....	0
23	Fast				....	....	2.84	+	....	268	....	0
24	Fast	51	26	804	....	....	2.76	+	....	300	....	0
25	60	"	"	"	....	....	5.13	13.68	....	268	57.6	0
26	"	"	"	"	....	....	5.70	21.80	....	....	....	0
27	"	"	"	"	....	....	1.88	15.58	....	....	....	0
28	"	"	"	"	....	....	2.88	0	....	....	....	8
Mar.												
1	"	"	"	"	....	....	2.92	0	....	171	....	8
2	"	"	"	"	....	....	3.03	0	....	....	....	8
3	"	"	"	"	....	....	6.79	0	....	....	....	8
4	60	113	60	1497	....	....	2.52	10.98	....	....	....	8
5	"	"	"	"	....	....	3.12	11.13	....	....	....	8
6	"	"	"	"	....	....	4.81	+	....	312	....	8
7	"	"	"	"	....	....	2.50	7.03	....	....	....	8
8	"	"	"	"	....	....	2.18	7.65	....	....	....	12
9	"	"	"	"	....	....	4.64	12.30	....	441	....	12
10	60	80	60	1200	....	....	2.86	6.60	....	....	....	12
11	"	"	"	"	....	....	3.67	+	....	....	....	16
12	20	0	0	80	....	....	2.82	7.75	....	319	....	0
13	"	"	"	"	....	....	2.18	0	....	....	....	0
14	"	"	"	"	....	....	2.73	0	....	213	....	$1\frac{1}{2}$
15	"	"	"	"	....	....	2.03	0	....	175	....	$1\frac{1}{2}$
16	20	35	0	400	....	....	2.62	0	....	140	....	0
17	"	"	"	"	....	....	3.16	0	....	....	....	0
18	"	"	"	"	....	....	2.40	0	....	....	....	0
19	"	"	"	"	....	....	2.42	0	....	192	....	0

22	"	"	"	"	"	"	2 29	0	.....	203	.....	39	1
23	"	"	"	"	"	"	3 12	0	.....	217†	.....	42	1
24	"	"	"	"	"	"	2 25	0	.....	187	.....	.....	2
25	"	"	"	"	"	"	2 35	0	.....	136†	.....	40	2
26	"	"	"	"	"	"	1 79	0	.....	152	.....	.....	2
27	"	"	"	"	"	"	2 65	0	.....	125†	.....	40	1
28	"	"	"	"	"	"	2 36	0	.....	122	.....	.....	1
29	20	4	0	114	.....	.....	2 37	0	.....	180	.....	40	1
30	"	"	"	"	.....	.....	3 16	0	.....	136†	.....	.....	1
31	"	"	"	"	.....	.....	3 23	0	.....	178	.....	.....	1
April													
1	20	7	0	143	.....	.....	2 75	0	.....	150	.....	40	1
2	"	"	"	"	.....	.....	.....	0	.....	138†	.....	.....	1
3	"	"	"	"	.....	.....	2 90	0	.....	122†	.....	40	1
4	20	35	0	400	.....	.....	2 78	0	.....	160	.....	.....	1
5	"	"	"	"	.....	.....	2 30	0	.....	85†	.....	.....	1
6	"	"	"	"	.....	.....	2 30	0	.....	178	.....	.....	1
7	"	"	"	"	.....	.....	.....	0	.....	97†	.....	40	1
8	"	"	"	"	.....	.....	3 20	0	.....	132	.....	.....	1
9	"	"	"	"	.....	.....	2 90	0	.....	129†	.....	.....	1
10	15	60	0	600	.....	.....	2 40	0	.....	132	.....	40	1
11	"	"	"	"	.....	.....	1 50	0	.....	97†	.....	.....	1
12	"	"	"	"	.....	.....	1 90	0	.....	159	.....	40	1
13	"	"	"	"	.....	.....	1 80	0	.....	105†	.....	.....	1
14	"	"	"	"	.....	.....	1 90	0	.....	138	.....	.....	1
15	"	"	"	"	.....	.....	2 50	0	.....	112†	.....	40	1
16	60	151	100	2000	.....	.....	3 40	8 00	.....	150	.....	.....	1
17	"	"	"	"	.....	.....	4 30	25 00	.....	101†	.....	40	8
					.....	.....			.....	145	.....	.....	8
					.....	.....			.....	161†	.....	.....	
					.....	.....			.....	209	.....	40	1
					.....	.....			.....	127†	.....	.....	1
					.....	.....			.....	206	.....	.....	1
					.....	.....			.....	110†	.....	40	1
					.....	.....			.....	150	.....	.....	1
					.....	.....			.....	132†	.....	.....	1
					.....	.....			.....	193	.....	.....	1
					.....	.....			.....	125†	.....	40	1
					.....	.....			.....	250	.....	.....	1
					.....	.....			.....	.....	.....	40	8
					.....	.....			.....	.....	.....	40	8

\*At Russell Sage Institute from Jan. 5 to Feb. 6.

†At Russell Sage Institute from Nov. 15 to Dec. 26.

‡Blood sample taken at 7 pm. Other blood samples taken before breakfast.

free on the unchanged diet, and even with 8 units, marked hyperglycemia and a trace of glycosuria were present on Sept. 13.

September 14 to October 2, the diet was changed from the former 60 gm. protein, 60 gm. fat, 6 gm. carbohydrate and 804 calories, to the same protein and carbohydrate with only 10 gm. of fat, making only 354 total calories. It will be observed that the plasma sugar thus fell steadily to 0.137 per cent.

Though much of the fat withdrawn from the diet in the above change must have been replaced by combustion of body fat, beginning Oct. 3 the carbohydrate was increased by 5 gm., in order to replace the supposed glucose equivalent of the 50 gm. of fat in the original diet. The diet thus became 60 gm. protein, 10 gm. fat, 11 gm. carbohydrate and 374 calories. On this diet the plasma sugar by Oct. 6 had fallen to 0.115 per cent. Slight hyperglycemia resulted when the insulin was then reduced to 6 units daily, and glycosuria appeared promptly when insulin was omitted on Oct. 18. With continuance of the 6 units, hyperglycemia steadily fell, and with a return to 8 units on Oct. 29, the plasma sugar was down to 0.091 per cent. on the morning of Oct. 31 and hypoglycemic symptoms appeared that evening.

Beginning Nov. 1, a return to the original diet of 60 gm. protein, 60 gm. fat, 6 gm. carbohydrate and 804 calories caused hypoglycemic symptoms to remain absent, and by Nov. 14 the plasma sugar had risen to 0.178 per cent. Notice should be taken of the prevention of hypoglycemic symptoms in this and several other cases by the giving of fat.

The patient was then transferred to the Russell Sage Institute, from which he returned on Dec. 26 with glycosuria present. Marked hyperglycemia and occasional glycosuria persisted on this diet with 8 units of insulin daily up to Jan. 17.

Beginning Jan. 18, the carbohydrate was increased by 20 gm. and the fat reduced by 9 gm., so as to keep the total calories unchanged. For unknown reasons, sugar to the amount of 5 or 6 gm. was excreted on Jan. 23 and 25. With these exceptions, the change proved wholly beneficial, in that the plasma sugar fell progressively and the urine became completely sugar-free up to Feb. 7.

Withdrawal of insulin then resulted in marked glycosuria. Mention has been made in paper 1 of the patient's gain of tolerance, as indicated by the positive carbohydrate balance at this time, and the results of the fasting period Feb. 21-24 and the low diet period March 12-15. In view of the length of the record and the controls used in the experimental tests, it is believed that this alteration of tolerance does not invalidate the results.

The first test, beginning Sept. 14, proved definitely that the tendency to hyperglycemia and glycosuria was less with a diet of reduced calories, as compared with the original diet of 804 calories which was given both before and afterward. The second test with the addition of 20 gm. carbohydrate, beginning Jan. 18, failed to show any increased glycosuria from this quantity of carbohydrate when the total calories were kept the

same by reduction of fat. The entire experience is opposed to the idea that the glucose value of the diet is the essential determining factor.

A still stronger proof of the gain of tolerance was furnished by the continued absence of glycosuria on a diet of 400 calories without insulin up to March 22. It will be observed that with only 20 gm. protein from March 12 onward, the plasma sugar had fallen steadily to 0.140 per cent. on the morning of March 16. The introduction of 35 gm. of fat, raising the total calories to 400, caused a steady rise of plasma sugar to 0.203 per cent. on the morning of March 22. In the former period, the use of body fat probably made up a metabolism in the neighborhood of 400 calories, and this experiment gives one more illustration of the difference between undernutrition and a "basal" diet. It was evident that glycosuria would soon occur, and that the patient's tolerance was slightly less than 400 calories. The deficit was not great, as in the period up to March 29 one unit of insulin seemed to suffice and 2 units was excessive as indicated by evening hypoglycemia.

March 29 to April 3, the diet was made nearly fat-free, with a view to demonstrating hypoglycemia from the small dosage of insulin under these circumstances. There was a falling tendency in the evening plasma sugars, as shown, but it was considered undesirable to prolong the experiment for the length of time evidently necessary for a decisive result. April 4 to 9, the restoration of fat to make 400 calories confirmed the above estimate that one unit of insulin sufficed for the assimilation of this diet.

April 10 to 15, the protein was reduced to 15 gm. and the fat increased so as to raise the total calories to 600. The glucose value of the diet was thus scarcely altered, but there was a distinct reduction of protein catabolism, as indicated by the urinary nitrogen. The plasma sugar nevertheless rose perceptibly as compared with the former period. In view of the improved tolerance, a long period of time would doubtless have been required for this small addition of fat to result in glycosuria. The small results of the small changes, however, conform fully to the rule that the assimilation is affected by the total calories and not merely by the glucose entering into metabolism with a given diet.

With a change of diet to 60 gm. protein, 100 gm. carbohydrate and 2000 calories, beginning April 16, the insulin requirement has proved to be approximately 28 units per day (not shown in table). Undoubtedly this requirement will rise as the body weight increases. The relation of this dosage to the undernourished state, and the comparison with case No. 989, are discussed under Section 5 at the close of this paper.

#### REMARKS ON TABLE 12.

This patient had shown constantly subnormal plasma sugars on a semi-starvation regime with 1 unit of insulin per day, and on August 23 had been in dangerous hypoglycemic collapse with only  $\frac{1}{2}$  unit. Though 156 gm. of carbohydrate was necessary to revive him on this day, it was proposed to use fat as a means of preventing further attacks of this kind. Beginning August 24, the protein ration was 25 gm. and the carbohydrate

TABLE 12  
Case No. 1194

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Aug.									
20	20	4	25	216	0	8.49	$\left\{ \begin{array}{l} 77 \\ 91^{\dagger} \\ 61^* \end{array} \right\}$	100	1
21	"	"	"	"	0	8.30	$\left\{ \begin{array}{l} \dots \\ 55 \end{array} \right\}$	...	1
22	25	7	40	323	0	8.52	$\left\{ \begin{array}{l} 69^{\dagger} \\ 53^* \\ 46^{\dagger} \end{array} \right\}$	...	1
23			156		0	9.09	$\left\{ \begin{array}{l} 42^{\dagger} \\ 57^{\dagger} \\ 75^{\dagger} \end{array} \right\}$	...	$\frac{1}{2}$
24	25	203	15	2000	0	9.00	$\left\{ \begin{array}{l} 150^{\dagger} \\ 270 \\ 312^{\dagger} \\ 220^* \end{array} \right\}$	...	..
25	25	250	15	2410	0	5.15	$\left\{ \begin{array}{l} 230 \\ 306^{\dagger} \\ 254^* \end{array} \right\}$	100	$\frac{1}{2}$
26	"	"	"	"	0	4.70	$\left\{ \begin{array}{l} 300 \\ 270^{\dagger} \end{array} \right\}$	...	1
27	"	"	"	"	0	2.92	$\left\{ \begin{array}{l} 238^* \\ 254 \\ 288^{\dagger} \end{array} \right\}$	...	1
28	"	"	"	"	0	3.00	$\left\{ \begin{array}{l} \dots \\ 214^* \end{array} \right\}$	102	1
29	"	"	"	"	0	2.24	254	...	1
30	25	300	10	2840	0	3.07	...	...	1
31	"	"	"	"	0	2.67	...	...	1
Sept.									
1	"	"	"	"	0	2.75	206	109	1
2	"	"	"	"	0	3.02	...	...	1
3	"	"	"	"	0	2.29	230	107	1
4	"	"	"	"	0	1.96	214 <sup>†</sup>	...	1
5	25	300	10	2840	0	1.06	226	107	1
6-7	"	"	"	"	0	2.10	...	...	1
8	"	"	"	"	0	2.34	270	104	1
9	"	"	"	"	0	2.56	...	103	1
10	"	"	"	"	0	2.34	280*	...	1
11	"	"	"	"	0	2.60	257	106	1
12	"	"	"	"	0	1.88	...	...	1
13	"	"	"	"	0	2.85	278*	...	1
14	25	0	40	260	0	3.71	258	...	1
15-17	"	"	"	"	0	3.05	...	101	1
18	"	"	"	"	0	2.98	$\left\{ \begin{array}{l} 268 \\ 192^* \end{array} \right\}$	...	1
19-22	"	"	"	"	0	2.80	...	104	1
23	"	"	"	"	0	3.62	206	105	1
24	"	"	"	"	0	3.71	156	...	1
25	"	"	"	"	0	4.32	...	107	1
26	"	"	"	"	0	3.54	$\left\{ \begin{array}{l} 160 \\ 192^* \end{array} \right\}$	106	1

†Blood sample taken at 3 p.m.

\*Blood sample taken at 8 p.m.

<sup>1</sup>9.30 a.m.<sup>2</sup>11.00 a.m.<sup>3</sup>1.00 p.m.<sup>4</sup>2.15 p.m.<sup>5</sup>6.15 p.m.

TABLE 12.—*Continued*

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Sept.									
27	25	0	40	260	0	4.11	...	...	1
28	"	"	"	"	0	4.28	162	...	1
29	"	"	"	"	0	4.10	112	106	1
30	"	"	"	"	0	4.80	139*	...	1
Oct.									
1	25	300	10	2840	0	3.40	93	106	1
2	"	"	"	"	0	2.70	118*	...	1
3	"	"	"	"	0	3.83	157	106	1
4-5	"	"	"	"	0	2.94	...	107	1
6	"	"	"	"	0	2.93	187*	...	1
7-9	"	"	"	"	0	2.54	...	108	1
10	"	"	"	"	0	2.09	187	...	1
11-13	"	"	"	"	0	2.57	...	109	1
14	"	"	"	"	0	2.51	217	...	1
15	"	"	"	"	0	1.14	...	109	1
16	100	10	10	530	0	1.09	230	...	1
17	"	"	"	"	0	8.73	...	109	1
18	"	"	"	"	0	11.78	217	...	1½
19	"	"	"	"	0	14.33	226*	113	1
20-21	"	"	"	"	0	12.10	...	...	1
22	"	"	"	"	0	12.77	{ 234 230* }	115	1
23-25	"	"	"	"	0	9.67	...	113	1
26	"	"	"	"	0	8.03	{ 217 182* }	...	1
27-29	"	"	"	"	0	12.61	...	109	1
30	"	"	"	"	0	12.08	{ 160 159* }	...	1
31	"	"	"	"	0	13.33	...	109	1
Nov.									
1	"	"	"	"	0	14.19	...	...	1
2	"	"	"	"	0	17.89	154	105	1
3-5	"	"	"	"	0	14.80	...	103	1
6	"	"	"	"	0	15.49	{ 109 84* }	105	1
7	"	"	"	"	0	16.48	...	...	1½
8	100	62	10	1000	0	15.67	103	105	1
9-10	"	"	"	"	0	15.87	...	106	1
11	"	"	"	"	0	16.61	...	172	1
12-13	"	"	"	"	0	11.80	...	107	1
14	"	"	"	"	0	16.51	...	103	1
15	"	"	"	"	0	12.10	187	...	1
16	100	117	10	1500	0	12.91	...	...	1
17	"	"	"	"	0	12.55	230	...	1
18-21	"	"	"	"	+	14.20	...	110	1
22	"	"	"	"	++	14.40	...	...	1½
23	"	"	"	"	+++	13.28	334	111	1
24	"	"	"	"	++++	16.17	...	110	1
25	"	"	"	"	++	15.39	395	...	2½
26	"	"	"	"	0	17.11	283*	109	4
27	"	"	"	"	+	13.68	...	...	4
28	"	"	"	"	++	13.29	334	110	2
29	"	"	"	"	++	10.81	...	...	2

\*Blood samples taken at 8 p.m.



TABLE 12.—Continued

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Nov. 30	100	117	10	1500	+	11.67	...	110	4
Dec. 1	"	"	"	"	++	...	300	...	4
2-3	"	"	"	"	+	12.85	...	110	4
4	"	"	"	"	8.29	7.20	...	108	4
5	"	"	"	"	64.96	15.91	300	...	4
6	"	"	"	"	27.43	12.78	...	199	4
7	"	"	"	"	18.53	14.23	...	...	4
8	"	"	"	"	16.40	16.40	...	110	4
9	"	"	"	"	...	11.93	...	...	4
10	"	"	"	"	54.45	19.60	...	111	4
11	"	"	"	"	28.52	13.36	...	...	4
12	"	"	"	"	37.70	18.00	...	111	8
13	"	"	"	"	49.47	18.01	405	...	8
14	"	"	"	"	63.90	22.18	...	112	8
15	"	"	"	"	36.31	15.94	...	...	8
16	"	"	"	"	...	11.34	416	112	8
17	"	"	"	"	25.31	13.90	...	...	8
18	"	"	"	"	43.78	15.15	...	115	8
19	"	"	"	"	...	...	469	...	8
20-25	100	100	25	1400	...	...	...	...	12
26-27	"	"	"	"	...	...	...	...	...
28	"	"	"	"	...	...	...	...	12
29	"	"	"	"	33.59*	8.18	429	...	12
30	"	"	"	"	82.00	17.60	...	112	14
31	"	"	"	"	34.15	21.84	...	...	16
1923 Jan.									
1	"	"	"	"	27.59	13.69	375	112	16
2	"	"	"	"	22.27	9.04	...	...	16
3	"	"	"	"	25.67	10.70	...	112	16
4	"	"	"	"	25.21	13.10	...	...	16
5	"	"	"	"	...	13.11	405	111	16
6	"	"	"	"	36.81	15.10	...	...	16
7	"	"	"	"	13.80	13.20	...	110	20
8	"	"	"	"	15.06	11.64	455	...	20
9	"	"	"	"	43.26	16.38	...	108	20
10	"	"	"	"	...	16.38	...	...	24
11	"	"	"	"	24.99	20.05	...	110	24
12	"	"	"	"	41.28	22.73	455	...	24
13	"	"	"	"	29.64	17.68	...	110	24
14	"	"	"	"	90.00	20.70	...	...	30
15	"	"	"	"	52.08	20.06	405	111	30
16	"	"	"	"	71.08	18.03	...	...	30
17	"	"	"	"	66.15	19.65	...	112	30
18	"	"	"	"	40.88	18.27	405	...	36
19	"	"	"	"	94.72	18.95	...	112	36
20	"	"	"	"	4.90	17.05	416	...	36
21	"	"	"	"	4.83	12.17	...	109	40
22	"	"	"	"	42.00	19.09	441	...	50
23	"	"	"	"	31.75	20.86	385	114	50
24	"	"	"	"	4.95	6.75	385	...	50

\* Blood sample taken at 5 p.m.



TABLE 12.—Continued

Date	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
1923									
Jan.									
25	100	100	25	1400	6 12	19 65	...	113	50
26	"	"	"	"	6 48	14 50	{ 357 154* }	...	50
27	"	"	"	"	56 70	19 74	...	111	50
28	90	160	50	2000	—	13 44	...	...	50
29	"	"	"	"	22 40	13 48	...	115	50
30	"	"	"	"	14 37	18 67	90*	...	50
31	"	"	"	"	0	...	...	110	50
Feb.									
1	100	197	80	2500	0	14 36	...	...	50
2	"	"	"	"	+	12 32	272	...	50
3	"	"	"	"	19 36	11 64	...	...	50
4	"	"	"	"	11 28	9 40	189*	113	50
5	"	"	"	"	7 95	10 11	312	...	50
6	"	"	"	"	7 25	10 78	97*	114	50
7	"	"	"	"	0	12 82	334	...	50
8	90	215	50	2500	0	11 59	272	115	50
9	"	"	"	"	+	8 69	...	...	50
10	"	"	"	"	+	9 58	306	115	50
11	90	171	150	2500	7 59	...	...	...	50
12	"	"	"	"	0	...	...	115	50
13	"	"	"	"	0	...	272	...	50
14	"	"	"	"	0	...	...	117	50
15	90	171	200	2700	0	...	166	...	50
16	"	"	"	"	+	...	...	117	50
17	"	"	"	"	55 88	...	395	...	50
18	"	"	"	"	30 38	...	...	117	50
19	"	"	"	"	18 50	...	...	...	50
20	"	"	"	"	++++	...	...	117	50
21	100	222	150	3000	31 95	...	349	...	50
22	"	"	"	"	27 36	...	...	117	50
23	"	"	"	"	6 72	...	...	...	50
24	"	"	"	"	19 24	...	341	118	50
25	"	"	"	"	6 85	...	...	...	54
26	"	"	"	"	14 30	...	...	118	54
27	"	"	"	"	34 60	...	341	...	54
28	100	277	150	3500	30 26	...	...	120	54
Mar.									
1	"	"	"	"	++	...	...	...	54
2	"	"	"	"	35 26	...	...	120	54
3	"	"	"	"	14 98	...	366	120	54
4	"	"	"	"	31 08	...	395	119	54
5	"	"	"	"	9 72	...	226	...	54
6	"	"	"	"	20 48	...	375	121	54
7	"	"	"	"	46 05	...	...	...	54
8	"	"	"	"	42 80	...	...	122	54
9	"	"	"	"	++++	...	...	...	54
10	"	"	"	"	++++	...	455	122	54
11	"	"	"	"	31 08	12 42	416	...	54
12	100	20	200	1380	++++	14 92	441	122	54
13	"	"	"	"	29 43	12 06	...	...	54
14	"	"	"	"	72 30	19 62	341	119	54
15	"	"	"	"	72 50	21 42	259	...	54

\* Blood samples taken at 8 p.m.

TABLE 12.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Mar.									
16	100	20	200	1380	85.80	12.90	349	123	54
17	100	20	175	1280	0	9.57	...	...	54
18	"	"	"	"	0	10.55	...	122	54
19	"	"	"	"	0	13.68	294	...	54
20	100	20	200	1380	35.20	15.34	...	121	54
21	"	"	"	"	39.40	13.18	306	...	54
22	"	"	"	"	38.25	11.47	273	123	54
23	100	20	175	1280	++	10.93	...	...	54
24	"	"	"	"	0	9.68	...	...	54
25	"	"	"	"	3.99	8.53	268	...	54
26	"	"	"	"	0	9.88	...	123	54
27	"	"	"	"	12.98	14.85	300	...	54
28	"	"	"	"	24.20	14.83	...	123	54
29	"	"	"	"	++	11.73	312	...	40
30	"	"	"	"	29.80	...	...	120	40
31	"	"	"	"	0	...	...	...	40
April									
1	"	"	"	"	26.60	...	...	120	44
2	"	"	"	"	31.80	...	...	...	44
3	"	"	"	"	+++	...	385	120	44
4	"	"	"	"	50.10	...	...	...	44
5	100	70	125	1530	33.00	...	385	122	44
6	"	"	"	"	38.20	...	...	...	44
7	"	"	"	"	9.50	...	...	118	44
8	100	100	95	1680	4.20	...	...	...	44
9	"	"	"	"	14.90	...	...	118	44
10	"	"	"	"	+	...	...	...	44
11	"	"	"	"	6.80	...	...	117	44
12	"	"	"	"	4.20	...	263	...	44
13	"	"	"	"	—	...	...	117	44
14	"	"	"	"	0	...	...	...	44

15 gm., representing slightly less glucose than the 20 gm. protein and 25 gm. carbohydrate on August 20 and 21. An allowance of 10 per cent. glucose in the 250 gm. of fat which was given would still raise the total glucose value of the diet no higher than with the 25 gm. protein, 40 gm. carbohydrate and 7 gm. fat which failed to prevent hypoglycemia on August 22. Hyperglycemia was present at the beginning of the high fat regime, because of the 156 gm. carbohydrate given on August 23. It will be observed that this hyperglycemia persisted, and symptoms of low blood sugar remained entirely absent on the high fat diet. Beginning August 30, the fat was increased to 300 gm., and the carbohydrate was reduced to 10 gm., so as to raise the total calories without altering the theoretical glucose value of the diet. The hyperglycemia increased up to Sept. 13, and there is no doubt that glycosuria would have followed had the program been continued for a sufficient length of time.

September 14 to 30, the protein was kept unchanged at 25 gm., fat was

excluded completely, and carbohydrate was increased to 40 gm. so as to maintain the same theoretical glucose value of the diet while reducing the calories. The plasma sugar steadily fell and became normal at the end of this period, which had to be terminated in order to prevent hypoglycemia.

The opposite change was then made, reducing the carbohydrate to 10 gm. and restoring the 300 gm. fat and 2840 total calories. The plasma sugar by Oct. 16 had risen to 0.230 per cent., and it was again evident that glycosuria would follow if sufficient time were taken to allow the slow influence of fat to become fully manifest.

Beginning Oct. 16, the diet was changed to 100 gm. protein, 10 gm. fat, 10 gm. carbohydrate, and 530 calories. The theoretical glucose value was thus not greatly changed, but a test of the influence of protein was thus planned by giving the greater part of the total calories in the form of protein. With the undernutrition, the plasma sugar fell as usual and reached normal at the close of this period, Nov. 8.

November 8-15, the diet was raised to 1000 calories by increasing the fat to 62 gm. daily, which was supposed to be no more than the patient was burning from his own body fat on the low diet. On this "basal" ration the plasma sugar rose to 0.187 per cent. on Nov. 15.

November 16-December 19, the fat was increased to 117 gm., so as to raise the total calories to 1500 daily. Glycosuria quickly appeared, and toward the end of the period ranged from 25 to 64 gm., notwithstanding increase of the insulin dosage to 8 units daily. It seems self-evident that this effect of the fat is out of all proportion to any influence that could be attributed to the 11.7 gm. of glucose theoretically represented in its glycerol.

In the subsequent period, it will be noticed that the diet was gradually increased until at the beginning of February it amounted to 100 gm. protein, 80 gm. carbohydrate and 2500 calories. Glycosuria on this diet was not fully controlled by 50 units of insulin divided into four doses each day. It seems self-evident that this quantity of insulin would not have been required had the patient received no food except 150 gm. carbohydrate, which represented approximately the glucose value of the above diet.

February 8 to 20, changes were made in the carbohydrate content of the diet, first by reducing it to 50 gm., and then raising it to 150 and to 200 gm. The first changes showed surprisingly little effect. The increase to 200 gm. carbohydrate caused glycosuria, especially as the fat was not decreased and therefore the total calories were raised.

Beginning Feb. 21, the protein was changed to an unimportant extent by raising it to 100 gm. Carbohydrate was reduced to 150 gm. daily. Fat was increased so as to raise the total calories to 3000 and then to 3500 daily. This diet had a slightly lower glucose value than the preceding one, but heavy glycosuria persisted up to March 11, notwithstanding an increase of insulin to 54 units daily.

March 12-16, the diet was changed to 100 gm. protein, 20 gm. fat, 200 gm. carbohydrate, and 1380 calories. The glucose value was higher than

that of the preceding diet, and glycosuria showed a decided increase during this period notwithstanding the lower calories. For the purpose of a fairer test, the carbohydrate was then reduced to 175 gm., to correspond more accurately to the glucose value of the former 3500 calory diet. There was a surprising effect, in that glycosuria cleared up immediately and remained absent during the three days, March 17-19, of this diet. A return to 200 gm. carbohydrate during the following three days, March 20-22, again resulted in glycosuria which exceeded the 25 gm. difference in carbohydrate intake. With another reduction to 175 gm. carbohydrate, glycosuria was mostly absent from March 23 to 26, but returned heavily on March 27 and 28. In this experiment the glycosuric effect of pre-formed carbohydrate in the diet was unusually strong, and on the whole it counterbalanced the influence of the sharp reduction of calories. Nevertheless, the slower influence of the reduction of fat became more decisively evident, so that by March 31 the patient had become completely sugar-free on a reduced dosage of 40 units of insulin per day.

This reduced dosage, and also a slight increase to 44 units, failed to keep glycosuria absent. An experiment was then performed of interchanging carbohydrate and fat gram for gram. Beginning April 5, the carbohydrate was reduced by 50 gm. and the fat increased to 70 gm., so as to raise the total calories from 1280 to 1530. Glycosuria fell markedly. April 8, the carbohydrate was further reduced by 30 gm. and the fat increased by 30 gm., raising the total calories to 1680. The glycosuria thus fell almost to the vanishing point.

All these experiments confirm the greater glycosuric effect of carbohydrate as compared with fat both calory for calory and gram for gram. This difference, however, is much less than would be anticipated by persons who have supposed that carbohydrate alone need be considered, and other phases of the experiment show the powerful influence which is exerted by fat.

#### REMARKS ON TABLE 13.

On a low ration of 20 gm. protein and 5 gm. carbohydrate with 1 unit of insulin per day, this patient had hyperglycemia but not glycosuria up to August 23.

August 24-29, fat was added to the amount of 150 gm. daily, raising the total calories to 1450. There was no glycosuria and no particular change in the hyperglycemia during this time. The case was evidently not sufficiently severe to show marked effects from this degree of dietary change, unless the experiment should be continued for an inconvenient length of time. Accordingly, this plan was discontinued and a fresh start made with a view to obtaining quicker results.

August 30 to Sept. 9, a fat-free diet of 20 gm. protein, 20 gm. carbohydrate and 160 calories was given. The theoretical glucose value was identical with that of the preceding diet. Hyperglycemia continued until an increase to 2 units of insulin, beginning Sept. 5, reduced it gradually to 0.125 per cent. by Sept. 9.

The carbohydrate was then substituted by 200 gm. of fat, keeping the

TABLE 13  
Case No. 1073

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Aug. 17	30	8	0	195	0	.....	{ 157 214† 209* 226	112	1
18	30	10	0	210	0	.....	{ 187† 217* 220	...	1
19	30	3	0	150	0	5 07	{ 230† 223* }	116	1
20	20	12	3	200	0	4 79	{ ... 242 }	...	1
21	20	5	5	145	0	5 00	{ 226† 166* }	119	1
22	"	"	"	"	0	6 31	{ ... }	...	1
23	"	"	"	"	0	6 25	{ 263† 154* 153	122	1
24	20	150	5	1450	0	5 74	{ 159† 117* 182	...	1
25	"	"	"	"	0	4 87	{ 178† 182* 189	121	1
26	"	"	"	"	0	4 03	{ 166† 178* }	...	1
27	"	"	"	"	0	3 53	{ 217 180† }	117	1
28	"	"	"	"	0	3 05	{ ... 195* }	115	1
29	"	"	"	"	0	3 29	{ ... 258 }	...	1
30	20	0	20	160	0	3 20	{ ... }	112	1
31	"	"	"	"	0	3 84	{ ... }	...	1
Sept. 1	"	"	"	"	0	4 80	{ 246 }	110	1
2	"	"	"	"	0	7 46	{ ... }	...	1
3	"	"	"	"	0	5 90	{ 209 223* }	108	1
4	"	"	"	"	0	5 51	{ ... }	...	1½
5	"	"	"	"	0	5 15	{ ... }	109	2
6	"	"	"	"	0	6 16	{ 203 214* }	109	2
7	"	"	"	"	0	5 86	{ ... }	...	2
8	"	"	"	"	0	6 64	{ 154 157* }	...	2
9	"	"	"	"	0	7 20	{ 125 171* }	...	2
10	20	200	0	1880	0	4 58	{ 126 150* }	109	2
11	"	"	"	"	0	4 21	{ 187 }	...	2
12	"	"	"	"	0	2 77	{ ... }	107	2
13	"	"	"	"	0	3 55	{ 120* }	...	2
14	"	"	"	"	0	2 90	{ 157 }	...	2

†Blood sample taken at 3 p.m.

\*Blood sample taken at 8 p.m.

Other blood samples taken before breakfast.

TABLE 13.—*Continued*

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Sept. 15-17	20	200	0	1880	0	3 20	...	107	2
18	"	"	"	"	0	3 41	{ 217 326* }	107	2
19-20	"	"	"	"	0	2 70	...	108	2
21	"	"	"	"	0	3 48	{ 214 195* }	...	2
22-25	"	"	"	"	0	3 09	...	109	2
26	"	"	"	"	0	2 84	{ 234 182* }	109	2
27-28	"	"	"	"	0	3 05	...	109	2
29	"	"	"	"	0	3 77	168*	...	2
30	"	"	"	"	0	3 00	187	...	2
Oct. 1-2	"	"	"	"	0	2 91	...	112	2
3	"	"	"	"	0	1 93	184*	112	2
4	"	"	"	"	0	...	223	...	2
5-8	"	"	"	"	0	2 65	...	111	2
9	"	"	"	"	0	3 09	{ 252 199* }	110	2
10-11	"	"	"	"	0	2 81	...	110	2
12	"	"	"	"	0	...	206*	...	2
13	"	"	"	"	0	4 35	238	110	2
14	"	"	"	"	0	4 31	254*	...	2
15	80	10	0	410	0	5 71	272	111	2
16-17	"	"	"	"	++	9 20	...	110	2
18	"	"	"	"	++	11 97	...	...	1
19	"	"	"	"	++	10 67	{ 349 357* }	110	2
20-21	"	"	"	"	++	4 92	...	110	2
22	"	"	"	"	++	3 44	{ 312 326* }	...	2
23-25	"	"	"	"	++	7 08	{ 375 294* }	...	2
26	"	"	"	"	++	5 90	...	...	2
27-29	"	"	"	"	+	12 10	{ 288 263* }	111	2
30	"	"	"	"	+	12 67	...	...	2
31	"	"	"	"	+	10 81	...	111	2
Nov. 1	"	"	"	"	+	14 75	...	...	2
2	"	"	"	"	+	12 84	{ 277 250* }	...	2
3-5	"	"	"	"	++	11 40	...	...	2
6	"	"	"	"	+	11 76	{ 272 272* }	112	2
7	40	10	24	346	+	10 60	...	...	2
8	"	"	"	"	+	8 50	268*	113	2
9	"	"	"	"	+	9 52	258	...	4
10-11	"	"	"	"	+	8 20	...	112	4
12-13	"	"	"	"	0	8 62	...	...	6
14	"	"	"	"	0	...	{ 195 182* }	110	6
15	"	"	"	"	0	...	...	...	6

\*Blood sample taken at 8 p.m.



TABLE 13.—Continued

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Nov.									
16	40	10	24	360	0	.....	156	112	6
17	"	"	"	"	0	.....	182	...	6
18	"	"	"	"	0	.....	182*	111	6
19	40	160	24	1696	0	.....	195	...	6
20-21	"	"	"	"	0	.....	...	113	6
22	"	"	"	"	++	.....	...	...	6
23	"	"	"	"	++	.....	312	112	6
24-27	"	"	"	"	++	.....	...	111	6
28-29	"	"	"	"	++	.....	...	113	6
30	"	"	"	"	+	.....	...	...	6
Dec.									
1	"	"	"	"	++	.....	326	...	6
2-5	"	"	"	"	++	.....	...	113	6
6	"	"	"	"	3.84	.....	...	113	6
7	"	"	"	"	6.66	.....	...	...	6
8	"	"	"	"	26.73	.....	...	113	6
9	"	"	"	"	26.03	.....	326*	...	6
10	"	"	"	"	57.83	.....	349	113	0
11	"	"	"	"	12.44	.....	...	...	5
12	"	"	"	"	18.64	.....	...	113	10
13	"	"	"	"	24.05	.....	...	...	10
14	"	"	"	"	36.21	.....	405	113	10
15	"	"	"	"	37.69	.....	...	...	10
16	"	"	"	"	.....	.....	375*	...	10
17	"	"	"	"	20.25	.....	469	...	14
18	"	"	"	"	4.25	.....	...	113	14
19	"	"	"	"	15.26	.....	...	...	14
20	"	"	"	"	20.24	.....	294*	114	14
21	"	"	"	"	37.59	.....	374	...	14
22	"	"	"	"	28.67	.....	...	114	16
23	"	"	"	"	15.37	.....	...	...	18
24	"	"	"	"	15.91	.....	...	...	18
25	"	"	"	"	.....	.....	...	...	18
26	"	"	"	"	.....	.....	...	116	18
27	"	"	"	"	4.76	.....	349	...	18
28	"	"	"	"	+	.....	...	116	18
29	"	"	"	"	10.92	.....	...	...	18
30	"	"	"	"	16.21	.....	...	117	18
31	"	"	"	"	14.25	.....	...	...	18
1923									
Jan.									
1	"	"	"	"	26.13	.....	...	117	18
2	"	"	"	"	11.62	.....	...	...	18
3	"	"	"	"	15.38	.....	...	118	18
4	"	"	"	"	10.69	.....	...	...	20
5	"	"	"	"	8.93	.....	306	116	20
6	"	"	"	"	12.01	.....	...	...	20
7	"	"	"	"	11.00	.....	349	116	22
8	"	"	"	"	10.69	.....	...	...	22
9	"	"	"	"	9.12	.....	...	116	22
10	"	"	"	"	20.49	.....	...	...	24

\*Blood sample taken at 8 p.m.



TABLE 13.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan.									
11	40	160	24	1696	18.80	.....	385	116	24
12	"	"	"	"	15.44	.....	...	...	24
13	"	"	"	"	10.01	.....	...	...	24
14	"	"	"	"	5.77	.....	...	...	28
15	"	"	"	"	+	.....	...	119	28
16	"	"	"	"	+	.....	319	...	28
17	"	"	"	"	+	.....	246	120	28
18	"	"	"	"	+	.....	...	...	32
19	"	"	"	"	0	.....	238	...	32
20	"	"	"	"	0	.....	...	...	32
21	"	"	"	"	0	.....	200	...	32
22	"	"	"	"	0	.....	...	...	32
23	"	"	"	"	0	.....	206	...	32
24	"	"	"	"	0	.....	...	...	32
25	"	"	"	"	0	.....	{ 76* 220 69* }	...	32
26	110	160	24	1976	0	.....	{ 254* 71* 300 76* 250 65* 250 82* }	...	21
27	240	160	24	2496	12.90	.....	{ 254* 71* 300 76* 250 65* 250 82* }	...	26
28	40	305	24	3000	+	.....	{ 250 65* 250 82* }	...	32
29	"	"	"	"	0	.....	{ 250 65* 250 82* }	117	32
30	"	"	"	"	+	.....	{ 250 65* 250 82* }	...	32
31	40	381	24	3687	.....	.....	...	...	32
Feb.									
1	40	416	24	4000	.....	.....	{ 76† 58* }	...	32
2	146	368	24	3994	0	7.57	254	119	32
3	200	348	24	4000	11.58	14.51	...	...	32
4	"	"	"	"	0	25.92	{ 173* 326 242† 171* }	...	32
5	"	"	"	"	91.47	18.95	{ 173* 326 242† 171* }	...	32
6	"	"	"	"	86.20	31.49	...	...	32
7	"	"	"	"	68.16	26.34	{ 211* }	...	32
8	300	50	24	1746	51.20	37.80	357	...	32
9	"	"	"	"	21.46	.....	{ 272* }	...	32
10	"	"	"	"	73.43	41.53	357	...	32
11	"	"	"	"	14.36	42.05	...	...	32
12†	"	"	"	"	7.23	44.69	...	...	32
13	"	"	"	"	6.47	38.82	{ 78* }	...	32
14	"	"	"	"	0	36.15	...	...	32
15	50	50	145	1230	.....	.....	...	...	32
16	"	"	"	"	.....	.....	{ 58† 68* }	...	32

†24 gm. carbohydrate given in addition to diet.

TABLE 13.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Feb.									
17	50	250	125	2950	0	7.88	312	...	21
18	"	"	"	"	+	...	...	...	32
19	"	"	"	"	18.14	5.33	...	124	32
20	"	"	"	"	47.52	12.53	250†	...	32
21	"	"	"	"	32.96	8.15	349†	...	32
22	"	"	"	"	22.05	5.88	...	...	32
23	"	"	"	"	21.78	6.64	...	...	32
24	"	"	"	"	...	4.98	...	...	32
25	"	"	"	"	9.50	4.38	306	...	32
26	"	"	"	"	10.53	5.37	...	...	32
27	"	"	"	"	8.28	5.09	...	...	32
28	"	"	"	"	+++	4.74	263	128	32
Mar.									
1	100	250	125	3150	++	6.09	...	...	32
2-4	"	"	"	"	0	7.61	...	...	32
5	"	"	"	"	12.76	11.04	...	127	32
6	"	"	"	"	13.25	11.13	...	...	32
7	"	"	"	"	8.25	8.58	...	...	32
8	"	"	"	"	23.20	12.20	334	128	32
9	"	"	"	"	44.30	13.96	...	...	32
10	"	"	"	"	38.70	15.90	...	129	32
11	"	"	"	"	21.18	11.85	300	...	32
12	125	20	150	1280	18.82	14.54	...	...	32
13	"	"	"	"	5.04	15.36	...	129	32
14	"	"	"	"	12.98	15.90	242	...	32
15	90	337	100	3500 <sup>s</sup>	9.80	14.55	202	...	32
16	"	"	"	"	9.60	12.15	...	...	32
17	"	"	"	"	63.00	10.72	...	129	32
18	"	"	"	"	19.75	10.62	...	...	32
19	"	"	"	"	30.50	10.48	...	129	32
20	"	"	"	"	29.80	10.30	...	...	32
21	"	"	"	"	78.60	12.93	375	129	32
22	"	"	"	"	29.10	11.29	...	...	32
23	"	"	"	"	39.10	12.26	...	129	32
24	"	"	"	"	43.50	11.24	...	...	32
25	"	"	"	"	20.25	11.69	...	...	40
26	"	"	"	"	32.00	11.00	...	129	40
27	"	"	"	"	18.92	11.18	...	...	40
28	"	"	"	"	19.80	10.09	...	...	40
29	"	"	"	"	33.70	11.40	...	133	40
30	"	"	"	"	26.30	9.50	...	...	40
31	"	"	"	"	32.90	10.90	...	132	40
April									
1	90	287	150	3550	50.50	11.60	...	...	40
2	"	"	"	"	+++	13.60	416	133	40
3	"	"	"	"	19.60	9.10	...	...	40
4	"	"	"	"	31.20	10.60	...	133	40
5	"	"	"	"	30.50	10.90	395	...	40
6	"	"	"	"	32.30	10.10	...	134	40
7	"	"	"	"	35.70	10.10	...	...	40
8	90	237	200	3300	42.80	12.40	...	134	40
9	"	"	"	"	41.80	10.30	...	...	40
10	"	"	"	"	63.00	10.50	...	134	40

†Blood sample taken at 3 p.m.

TABLE 13—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
April									
11	90	237	200	3300	75.00	11.40	...	...	40
12	"	"	"	"	47.50	11.30	441	135	40
13	"	"	"	"	33.00	12.00	...	...	40
14	"	"	"	"	49.70	12.50	...	136	40
15	"	"	"	"	82.20	12.80	...	...	40
16	"	"	"	"	.....	.....	469	...	40

theoretical glucose value unchanged, but raising the total calories to 1880. The plasma sugar slowly rose, and by the morning of Oct. 15 had reached 0.272 per cent.

October 15–November 6, a carbohydrate-free diet of 80 gm. protein, 10 gm. fat, and 410 calories was given. The theoretical glucose value was not greatly different from that of the preceding diet, but it was desired to test the influence of protein by giving most of the calories in this form. No allowance was made for the body fat which was necessarily burned during this period of low calories. Hyperglycemia increased, and glycosuria in small amounts was continuous. The glycosuric influence of the protein evidently overbalanced the opposing influence of the undernutrition.

November 7–18, the diet was 40 gm. protein, 10 gm. fat, 24 gm. carbohydrate and 346 calories. The glucose value was thus a trifle higher and the calories slightly lower than before, but it was desired especially to test the influence of the carbohydrate. As glycosuria continued on Nov. 7 and 8, the insulin was increased to 4 and then to 6 units. Glycosuria ceased and the plasma sugar fell, but did not reach a fully normal level at the end of this period.

Beginning Nov. 19, 150 gm. of fat was added without other change in the diet. Glycosuria quickly appeared, and was not halted until the insulin was gradually increased to 32 units on Jan. 18. Hypoglycemic collapse occurred with this program on Jan. 25. It is probable, therefore, that the actual insulin requirement was about 30 units per day. The reckoning then shows that 24 units of insulin was required to balance the addition of 150 gm. of fat to the diet in this instance.

On Jan. 26 and 27, the attempt was made to prevent hypoglycemia by means of protein. Though the total calories were also increased by this means, it is remarkable that neither 110 gm. nor 240 gm. of protein showed any perceptible influence in preventing the collapse.

An attempt was next made to overcome the hypoglycemia by means of fat. January 28 to February 1, the protein was reduced to the former quantity of 40 gm., the carbohydrate was left unchanged, and the patient ate as much fat as possible, thus attaining a ration of 4000 total calories.

on Feb. 1. Morning hyperglycemia and evening hypoglycemia continued as before. It seems probable that without the high fat the hypoglycemia would have become more severe, probably dangerous in degree, while with the fat it amounted only to uncomfortable weakness and perspiration for several hours each evening, without any imperative need for carbohydrate administration. The failure of fat to prevent hypoglycemia when given with the low protein and carbohydrate in this case is important theoretically, because it furnishes some evidence against the view that any considerable portion of the fat is converted into sugar.

February 2 to 7, the combined influence of high protein and high fat was observed. Here the protein served as a source of carbohydrate, and the high caloric value of the fat had its usual effect in lowering tolerance, so that the hypoglycemic symptoms quickly ceased and were replaced by hyperglycemia and heavy glycosuria.

February 8 to 14, the protein was further increased to 300 gm., while the fat was sharply reduced so as to lower the total calories to 1746. The glycosuria gradually cleared up, and on the evening of Feb. 12 such severe symptoms of hypoglycemia appeared that the giving of 24 gm. of extra carbohydrate was unavoidable. The influence of the fat in preventing hypoglycemia was thus made evident, and it was shown that the largest quantity of protein which the patient could eat was unable to prevent hypoglycemia with 32 units of insulin per day and a low caloric diet.

February 15 and 16, the protein was reduced by 250 gm., and carbohydrate was added so as to make the total theoretical glucose value slightly less than that of the preceding diet. The calories were thus reduced lower than before. Hypoglycemia still occurred, showing that this amount of preformed carbohydrate was inadequate to prevent it.

Beginning Feb. 17, a high diet of 50 gm. protein, 250 gm. fat, 125 gm. carbohydrate, and 2950 calories was given. A mild attack of grippe occurred about this time, but did not seem to alter the tolerance very appreciably. March 1 to 11, the protein was increased to 100 gm., raising the total calories because no changes were made in the carbohydrate or fat. The effect was probably an increase of glycosuria, but it is evident that the change was not great and probably no greater than might have resulted from an equal increase of calories in the form of fat.

March 12-14, the protein was increased to 125 gm. and the carbohydrate to 150 gm., while a radical reduction of the fat lowered the total calories to 1280. Here the influence of the undernutrition outweighed the increase of protein and carbohydrate, and resulted in a marked fall of glycosuria not only on these days but on the two following days, March 15 and 16.

March 15-31, the opposite change was made, by reducing the protein to 90 gm. and the carbohydrate to 100 gm., while an increase of fat to 337 gm. raised the total calories to 3800 per day. Glycosuria increased, and was not greatly reduced by an increase of insulin to 40 units beginning March 25. A characteristic of the glycosuria resulting from fat is that it is particularly difficult to overcome by either diet or insulin treatment.

April 1, an exchange of carbohydrate and fat on a gram for gram basis was begun. April 1-7, 50 gm. of fat was subtracted and 50 gm. of carbohydrate added. The influence upon the glycosuria was imperceptible. April 8-16, another exchange of 50 gm. was made, reducing the fat to 237 gm. and raising the carbohydrate to 200 gm. Notwithstanding the reduction of total calories thus involved, the greater glycosuric effect of the carbohydrate now became evident in a distinct increase of the sugar excretion. This increase, however, was small in comparison with the increase of carbohydrate intake, and illustrates the inaccuracy of attempts to estimate the insulin requirement by quantitative changes in a continuous glycosuria.

#### REMARKS ON TABLE 14.

This patient, with diabetes and nephritis, seemed to require between 3 and 6 units of insulin for the assimilation of a diet of 50 gm. protein, 10 gm. carbohydrate and 1000 calories up to Nov. 29.

November 30 to January 1, an increase of fat to 128 gm. raised the total calories to 1400. Hyperglycemia and glycosuria gradually resulted, and by Jan. 1 the sugar excretion amounted to 31.86 gm., notwithstanding an increase of insulin to 10 units daily. The increase of fat had amounted to only 44 gm., and it seems self-evident that the glucose content of this quantity of fat could not account for this result.

Up to Jan. 30, the insulin requirement for preventing glycosuria on a diet of 50 gm. protein, 30 gm. carbohydrate and 1400 calories was found to be approximately 33 units. The carbohydrate here was only 20 gm. higher than in the preceding diet. It is evident that this quantity of carbohydrate could not account for the great increase in insulin requirement. It is probable that the influence of this 20 gm. of carbohydrate was very slight, and that the increase of the insulin requirement from 6 units in November to 33 units in January was due chiefly to the increase of fat and total calories.

February 1-10, the carbohydrate was increased to 100 gm. without change in the protein or fat, so that the total calories were increased to 1680. There was no glycosuria and no perceptible influence upon the hyperglycemia, though such an influence might have become perceptible in the course of a longer time. In the next period, up to March 3, both fat and carbohydrate were increased so as to raise the total calories to 2213. Glycosuria began on Feb. 20, and became very heavy notwithstanding an increase of insulin to 40 units daily.

March 4-13, the carbohydrate was reduced to 75 gm. while the fat was increased to keep the total calories at 2213. Glycosuria diminished greatly, illustrating the stronger glycosuric effect of carbohydrate as compared with the caloric equivalent of fat. Nevertheless, it will be noticed that the insulin requirement on this diet with 75 gm. carbohydrate was distinctly higher than in the period Feb. 1-10 with 100 gm. carbohydrate and lower total calories.

Beginning March 14, the diet was changed to 60 gm. protein, 100 gm. carbohydrate and 2000 calories. The increase of carbohydrate and also



TABLE 14  
Case No. 24

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Nov.								
4	50	70	5	850	0	...	83	1/2
5	"	"	"	"	0	258	...	1
6	"	"	"	"	0	153	83	1
7	50	84	10	1000	0	133	...	2
8	"	"	"	"	0	138	82	2
9	"	"	"	"	0	...	...	2
10	"	"	"	"	0	125	82	2
11	"	"	"	"	0	...	...	2
12	"	"	"	"	0	128	83	2
13	"	"	"	"	0	...	...	2
14	"	"	"	"	0	164	80	2
15	"	"	"	"	0	...	...	2
16	"	"	"	"	0	153	80	4
17	"	"	"	"	0	169	...	4
18	"	"	"	"	0	...	80	4
19	"	"	"	"	0	...	...	6
20	"	"	"	"	0	...	80	6
21	"	"	"	"	0	171	...	6
22	"	"	"	"	0	...	80	3
23	"	"	"	"	0	...	...	6
24	"	"	"	"	0	...	78	6
25	"	"	"	"	0	211	...	6
26	"	"	"	"	0	...	79	6
27	"	"	"	"	0	195	...	6
28	"	"	"	"	0	...	...	3
29	"	"	"	"	0	214	...	3
30	50	128	10	1400	0	091	78	6
Dec.								
1	"	"	"	"	0	...	...	6
2	"	"	"	"	0	115	78	6
3	"	"	"	"	0	...	...	6
4	"	"	"	"	0	...	81	6
5	"	"	"	"	0	...	...	6
6	"	"	"	"	0	206	81	3
7	"	"	"	"	0	...	...	6
8	"	"	"	"	0	...	81	8
9	"	"	"	"	0	238	...	8
10	"	"	"	"	2.43	...	80	0
11	"	"	"	"	0	...	...	4
12	"	"	"	"	+	...	80	8
13	"	"	"	"	+	...	...	8
14	"	"	"	"	+	356	81	8
15	"	"	"	"	0	...	...	8
16	"	"	"	"	0	...	79	8
17	"	"	"	"	0	...	...	8
18	"	"	"	"	+	469	79	8
19	"	"	"	"	+	...	...	8
20	"	"	"	"	0	...	79	8
21	"	"	"	"	+	...	...	5
22	"	"	"	"	+	...	79	10
23	"	"	"	"	+	455	...	10
24	"	"	"	"	+	...	78	10
25	"	"	"	"	0	...	...	10



TABLE 14.—*Continued*

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Dec.								
26	50	128	10	1400	0	...	79	10
27	"	"	"	"	+	...	...	10
28	"	"	"	"	+	395	80	10
29	"	"	"	"	10.92	...	...	10
30	"	"	"	"	15.64	...	79	10
31	"	"	"	"	27.77	...	...	10
1923								
Jan.								
1	"	"	"	"	31.86	555	80	10
2	50	97	30	1200	42.49	...	78	10
3	"	"	"	"	28.19	...	...	10
4	"	"	"	"	37.00	...	...	10
5	"	"	"	"	41.82	536	79	10
6	"	"	"	"	40.27	...	...	10
7	50	120	30	1400	37.09	109	79	12
8	"	"	"	"	26.57	555	...	12
9	"	"	"	"	23.40	...	80	12
10	"	"	"	"	38.64	...	...	15
11	"	"	"	"	45.34	...	80	15
12	"	"	"	"	20.59	625	...	15
13	"	"	"	"	51.84	...	79	15
14	"	"	"	"	43.94	...	...	18
15	"	"	"	"	44.44	588	78	18
16	"	"	"	"	44.80	600	...	21
17	"	"	"	"	33.00	...	79	21
18	"	"	"	"	23.85	...	...	24
19	"	"	"	"	18.81	...	81	24
20	"	"	"	"	28.08	...	...	24
21	"	"	"	"	23.40	405	81	30
22	"	"	"	"	31.50	...	...	30
23	"	"	"	"	28.62	...	81	30
24	"	"	"	"	6.65	441	...	30
25	"	"	"	"	6.9	375	83	30
26	"	"	"	"	5.94	...	...	30
27	"	"	"	"	0	395	85	30
28	"	"	"	"	0	...	...	30
29	"	"	"	"	0	270	85	33
30	"	"	"	"	7.15	...	...	33
31	"	"	"	"	0	...	85	33
Feb.								
1	50	120	100	1680	+	...	...	33
2	"	"	"	"	0	...	85	33
3	"	"	"	"	8.60	270	...	33
4	"	"	"	"	0	...	86	33
5	"	"	"	"	0	220	...	33
6	"	"	"	"	0	...	88	33
7	"	"	"	"	0	226	...	33
8	"	"	"	"	0	...	90	33
9	"	"	"	"	0	...	...	33
10	"	"	"	"	0	263	90	33
11	50	157	150	2213	0	113	...	33
12	"	"	"	"	0	...	90	33
13	"	"	"	"	0	...	...	33
14	"	"	"	"	0	119	91	33
15	"	"	"	"	0	230	...	33
16	"	"	"	"	0	250	92	33

TABLE I-I.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.								
17-18	50	157	150	2213	0		92	33
19	"	"	"	"	0	214	...	33
20	"	"	"	"	+	258	90	33
21	"	"	"	"	41.07	...	...	33
22	"	"	"	"	58.75	577	...	33
23	"	"	"	"	41.58	...	...	33
24	"	"	"	"	42.12	...	...	33
25	"	"	"	"	40.47	555	...	40
26	"	"	"	"	44.40	...	86	40
27	"	"	"	"	40.40	577	...	40
28	"	"	"	"	54.00	...	86	40
Mar.								
1	"	"	"	"	72.00	...	...	40
2	"	"	"	"	52.80	600	86	40
3	"	"	"	"	31.54	...	...	40
4	50	190	75	2213	27.03	...	86	40
5	"	"	"	"	6.45	...	...	40
6	"	"	"	"	21.36	429	87	40
7	"	"	"	"	10.92	...	...	40
8	"	"	"	"	6.00	...	87	40
9	"	"	"	"	3.78	405	...	40
10	"	"	"	"	+	...	89	40
11	"	"	"	"	5.95	...	...	40
12	"	"	"	"	+	...	90	40
13	"	"	"	"	9.80	300	...	40
14	60	151	100	2000	+	334	91	40
15	"	"	"	"	4.13	...	...	40
16	"	"	"	"	+	...	92	40
17	"	"	"	"	+	...	...	40
18	"	"	"	"	3.64	...	93	40
19	"	"	"	"	0	...	...	40
20	"	"	"	"	3.28	334	93	40
21	"	"	"	"	0	300	...	40
22	"	"	"	"	0	...	93	40
23	"	"	"	"	0	349	...	40
24	"	"	"	"	0	...	93	40
25	"	"	"	"	0	300	...	40
26	"	"	"	"	0	121	95	40
27	"	"	"	"	0	175	...	32
28	"	"	"	"	0	...	96	32
29	"	"	"	"	0	...	...	32
30	"	"	"	"	0	...	98	32
31	"	"	"	"	0	199	...	32
April								
1	"	"	"	"	0	...	97	32
2	"	"	"	"	0	63	...	24
3	"	"	"	"	0	283	98	24
4	"	"	"	"	0	...	...	24
5	"	"	"	"	0	173	97	24
6	"	"	"	"	0	...	...	24
7	"	"	"	"	0	175	97	24
8	"	"	"	"	0	...	...	20
9	"	"	"	"	0	...	99	20
10	"	"	"	"	0	154	...	20

the rapidly rising body weight should have created an increased insulin requirement. Instead, glycosuria cleared up altogether, and it became possible to reduce the insulin dosage to 20 units per day. The impression is thus given that the tolerance of this patient for some reason increased considerably at this time, as sometimes happens under insulin treatment. The conclusiveness of the earlier observations showing the influence of fat and total calories is not affected.

#### REMARKS ON TABLE 15.

This patient became free from glycosuria on a fat-free diet of 25 gm. protein and 15 gm. carbohydrate with only 1 unit of insulin per day. On Sept. 25, it was necessary to omit the half unit of insulin which was ordinarily given in the evening and to give an extra 10 gm. of carbohydrate, in order to relieve hypoglycemic symptoms.

The opportunity was then taken to illustrate the prevention of hypoglycemia by fat feeding. For this purpose, all carbohydrate was withdrawn from the diet, and 150 gm. of fat was given, so as to keep the theoretical glucose value unchanged while raising the total calories to 1450. Trivial hypoglycemic symptoms occurred on the evening of the first day of this diet, Sept. 26, but thereafter all symptoms were absent. The plasma sugar rapidly rose, and by Oct. 2 it was evident that glycosuria was about to begin.

Fat was therefore omitted, and a diet of 25 gm. protein and 15 gm. carbohydrate reduced the plasma sugar to a subnormal level within four days. It is noticeable that the preformed carbohydrate in this diet gave rise to glycosuria during the first two days, Oct. 3 and 4, as not infrequently happens in patients who are thus changed when on the verge of glycosuria. On Oct. 7 it became necessary to omit the evening dose of insulin and to raise the diet to 60 gm. protein and 30 gm. carbohydrate in order to avert serious hypoglycemic symptoms. Insulin was then discontinued altogether, and low blood sugars persisted on the low calory diet up to Oct. 9. The burden placed upon the pancreatic function by the 150 gm. of fat in the former diet is thus made plainly evident.

The diet was then changed, so that by Oct. 14 it became 90 gm. protein, 40 gm. carbohydrate and 1500 calories. The higher carbohydrate value of this diet seemed to bring on glycosuria no more rapidly than the more exclusive fat ration which had been given at the end of September. The slight glycosuria which began on Oct. 16 was abolished by 2 units of insulin, beginning on Oct. 20. In order to check the rise of plasma sugar, it became necessary gradually to increase the insulin dosage to 12 units per day. With this dosage, a normal level of plasma sugar was reached at the close of this period, Dec. 2.

Beginning Dec. 3, the diet was raised to 1800 calories by increase of fat, keeping protein and carbohydrate the same. The plasma sugar rapidly rose, and from Dec. 10 onward there was marked and increasing glycosuria. At the close (Dec. 15), the urine contained about as many grams of glucose as the grams of fat by which the diet had been increased.

TABLE 15  
Case No. 328

Date	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin; Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
1922									
Sept.									
16	25	0	15	160	++++	.....	405	...	1
17	"	"	"	"	++++	.....	385*	109	1
18	"	"	"	"	++++	.....	416	...	1
19-20	"	"	"	"	++	.....	...	109	1
21-22	"	"	"	"	0	.....	...	108	1
23	"	"	"	"	0	.....	178	102	1
24	"	"	"	"	0	.....	...	...	1
25	"	"	25	200	0	.....	69	96	1 1/2
26	25	150	0	1450	0	.....	98	...	1
27	"	"	"	"	0	.....	254*	92	1
28	"	"	"	"	0	.....	...	...	1
29	"	"	"	"	0	.....	234	90	1
30	"	"	"	"	.....	.....	...	...	1
Oct.									
1	"	"	"	"	0	.....	349*	95	1
2	"	"	"	"	0	.....	336	...	1
3	25	0	15	160	++	.....	...	94	1
4	"	"	"	"	+	.....	...	...	1
5	"	"	"	"	0	.....	...	95	1
6	"	"	"	"	0	.....	106	...	1
7	60	0	30	360	0	.....	42*	90	1 1/2
8	"	"	"	"	0	.....	...	...	0
9	90	1	30	489	0	.....	80*	86	0
							95		
10	97	113	50	1600	0	.....	57† 129‡ 162§ 178	...	0
11	90	80	30	1200	0	.....	131	...	0
12	"	"	"	"	0	.....	129	...	0
13	"	"	"	"	0	.....	...	...	0
14	90	108	40	1500	0	.....	178	...	0
15	"	"	"	"	0	.....	...	...	0
16	"	"	"	"	+	.....	...	...	0
17	"	"	"	"	+	.....	319	85	0
18	"	"	"	"	++	.....	...	...	0
19	"	"	"	"	+	.....	349	87	1
20-21	"	"	"	"	0	.....	...	89	2
22	"	"	"	"	0	.....	306	...	2
23-25	"	"	"	"	0	.....	...	91	2
26	"	"	"	"	0	.....	288	...	2
27-30	"	"	"	"	0	.....	...	91	2
31	"	"	"	"	+	.....	226	91	2
Nov.									
1	"	"	"	"	0	.....	206	...	3
2-3	"	"	"	"	0	.....	...	93	3
4	"	"	"	"	0	.....	214	93	3
5-6	"	"	"	"	0	.....	...	97	5
7	"	"	"	"	0	.....	192	...	8
8	"	"	"	"	0	.....	...	...	8
9	"	"	"	"	0	.....	171	98	8
10-11	"	"	"	"	0	.....	...	98	8
12	"	"	"	"	0	.....	234	...	8
13	"	"	"	"	0	.....	246	...	8

†Blood sample taken at 10 a.m.

§Blood sample taken at 5 p.m.

\*Blood sample taken at 8 p.m.

TABLE 15.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Nov.									
14-15	90	108	40	1500	0	.....	...	98	8
16	"	"	"	"	0	.....	211	100	8
17-18	"	"	"	"	0	.....	...	99	8
19	"	"	"	"	0	.....	168	...	8
20-21	"	"	"	"	0	.....	...	100	8
22	"	"	"	"	0	.....	283*	100	6
23	"	"	"	"	0	.....	306	...	12
24-27	"	"	"	"	0	.....	...	101	12
28	"	"	"	"	0	.....	140	101	6
29	"	"	"	"	0	.....	...	101	6
30	"	"	"	"	0	.....	...	101	12
Dec.									
1	"	"	"	"	0	.....	117	...	12
2	"	"	"	"	0	.....	...	101	12
3	90	142	40	1800	0	.....	...	...	12
4	"	"	"	"	0	.....	187	100	12
5	"	"	"	"	0	.....	...	...	12
6	"	"	"	"	0	.....	206	100	12
7-8	"	"	"	"	0	.....	...	100	12
9	"	"	"	"	0	.....	294	...	12
10	"	"	"	"	19.28	3.55	...	101	12
11	"	"	"	"	19.75	10.29	...	...	12
12	"	"	"	"	19.46	19.50	...	102	12
13	"	"	"	"	35.89	8.91	518	...	12
14	"	"	"	"	31.82	12.01	...	100	12
15	"	"	"	"	35.22	12.97	...	...	12
16	"	"	"	"	++++	13.63	...	101	12
17	90	95	70	1500	31.11	8.79	577	...	12
18	"	"	"	"	31.11	12.92	...	101	12
19	"	"	"	"	16.95	15.48	...	...	12
20	"	"	"	"	13.90	13.47	...	101	12
21	"	"	"	"	14.16	15.46	484	...	12
22	"	"	"	"	23.14	11.17	...	101	12
23	"	"	"	"	20.39	7.16	...	...	12
24	"	"	"	"	.....	.....	518	102	12
25	"	"	"	"	16.21	10.35	...	...	12
26	"	"	"	"	17.55	11.94	...	102	12
27	"	"	"	"	50.95	12.58	484	...	12
28	"	"	"	"	57.52	11.15	...	102	12
29	"	"	"	"	48.38	10.35	...	...	12
30	"	"	"	"	63.20	15.64	...	102	12
31	"	"	"	"	70.68	17.83	...	...	12
1923									
Jan.									
1	"	"	"	"	++++	.....	500	102	12
2	"	"	"	"	31.50	.....	...	...	12
3	"	"	"	"	54.16	16.24	...	102	12
4	"	"	"	"	51.35	14.76	484	...	...
5	"	"	"	"	62.16	9.47	...	103	12
6	"	"	"	"	46.55	9.31	...	...	12
7	"	"	"	"	81.25	20.49	484	103	12
8	"	"	"	"	49.32	.....	...	...	12
9	"	"	"	"	70.40	.....	...	101	12
10	90	111	35	1500	43.14	13.45	518	...	12

\*Blood sample taken at 8 p.m.

TABLE 15.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan.									
11	90	111	35	1500	39.68	16.63	...	101	12
12	"	"	"	"	52.08	14.10	...	101	12
13	"	"	"	"	38.86	14.46	500	...	12
14	90	118	20	1500	115.25	...	...	102	12
15	"	"	"	"	111.60	24.39	...	...	12
16	"	"	"	"	105.00	20.74	...	...	12
17	"	"	"	"	86.40	18.64	405	102	12
18	"	"	"	"	71.76	11.72	...	...	12
19	"	"	"	"	150.88	20.66	...	102	12
20	"	"	"	"	107.88	21.55	...	...	12
21	"	"	"	"	103.12	21.52	455	102	12
22	"	"	"	"	99.50	20.16	...	...	12
23	"	"	"	"	115.20	23.48	...	104	12
24	"	"	"	"	29.12	18.11	455	...	12
25	"	"	"	"	28.71	12.29	...	104	12
26	"	"	"	"	24.97	15.49	...	...	12
27	"	"	"	"	32.90	16.95	...	104	12
28	"	"	"	"	+	11.76	...	...	12
29	"	"	"	"	+	11.43	326	102	12
30	"	"	"	"	30.37	12.81	...	...	12
31	"	"	"	"	0	15.71	258*	103	12
Feb.									
1	90	160	50	2000	23.76	9.40	366	...	12
2	"	"	"	"	62.40	14.00	455	105	12
3	"	"	"	"	82.10	6.13	...	...	12
4	"	"	"	"	68.82	15.45	...	104	12
5	"	"	"	"	57.75	15.52	...	...	12
6	"	"	"	"	30.15	7.18	...	104	12
7	"	"	"	"	14.56	13.72	366	...	12
8	90	20	150	1140	34.71	12.23	...	106	12
9	"	"	"	"	31.68	...	416†	...	12
10	"	"	"	"	67.84	14.10	429	106	12
11	"	"	"	"	42.07	16.42	...	...	12
12	"	"	"	"	70.56	17.83	...	107	12
13	"	"	"	"	63.00	13.69	441	...	12
14	"	"	"	"	69.30	15.28	...	108	12
15	"	"	"	"	...	...	405	...	12
16	"	"	"	"	62.16	16.80	...	107	12
17	"	"	"	"	42.00	14.11	199*	...	12
18	"	"	"	"	68.55	16.54	429	107	12
19	"	"	"	"	71.02	14.25	...	...	12
20	"	"	"	"	46.90	16.04	...	105	12
21	"	"	"	"	25.92	14.52	375	...	20
22	"	"	"	"	43.74	15.12	...	104	20
23	"	"	"	"	12.42	19.98	...	...	20
24	"	"	"	"	40.00	23.74	395	...	20
25	"	"	"	"	15.91	12.54	...	...	20
26	"	"	"	"	91.02	22.69	268	103	20
27	"	"	"	"	37.80	15.46	...	...	20
28	"	"	"	"	...	24.47	226	104	24
Mar.									
1	"	"	"	"	17.86	28.04	366	...	24
2	"	"	"	"	35.26	27.33	...	...	24
3	"	"	"	"	22.72	9.31	...	...	24
4	90	53	75	1140	16.95	15.22	...	105	24

†Blood sample taken at 3 p.m.

\*Blood sample taken at 8 p.m.



TABLE 15.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Mar.									
5	90	53	75	1140	16.00	18.73	...	...	24
6	"	"	"	"	6.46	18.97	341	104	24
7	"	"	"	"	+	16.07	...	...	24
8	"	"	"	"	0	15.02	270	102	24
9	"	"	"	"	0	14.95	...	...	24
10	"	"	"	"	0	11.72	203	102	24
11	90	20	150	1140	0	11.82	180	...	24
12	"	"	"	"	13.44	16.75	...	100	24
13	"	"	"	"	+	10.23	...	...	24
14	"	"	"	"	12.72	14.46	263	100	24
15	"	"	"	"	++	9.54	...	...	24
16	"	"	"	"	++	12.22	...	101	24
17	"	"	"	"	++	18.72	178	...	24
18	"	"	"	"	++	19.15	...	100	24
19	"	"	"	"	56 10	20 72	152	...	24

†Blood sample taken at 3 p.m.

\*Blood sample taken at 8 p.m.

Other blood samples taken before breakfast.

Beginning Dec. 17, carbohydrate was increased to 70 gm., while fat was decreased so as to reduce the total calories to 1500. Of these two opposing influences, the increase of carbohydrate proved more powerful than the reduction of calories, and glycosuria increased. Beginning Jan. 10, the carbohydrate was reduced to 35 gm., and on Jan. 14 to 20 gm., while fat was increased so as to keep the total calories at 1500. Glycosuria was evidently reduced by this change, though violations of diet at this time interfered with accurate results.

Beginning Feb. 1, both fat and carbohydrate were increased, so as to make a diet of 90 gm. protein, 160 gm. fat, 50 gm. carbohydrate, and 2000 calories. A distinct increase of glycosuria resulted. February 8, the carbohydrate was raised to 150 gm., while fat was sharply reduced to 20 gm., thus lowering the total calories to 1140. The glycosuria continued practically unchanged. The influence of the undernutrition, due to withdrawal of fat, was such as practically to counterbalance the great increase of carbohydrate.

Beginning Feb. 21, the attempt was made to abolish glycosuria by a gradual increase of insulin dosage. Up to March 3, an increase to 24 units per day on the same diet of 1140 calories had failed to reduce the glycosuria very greatly. Observations of this kind cast further doubt upon the accuracy of attempts to measure the effects of insulin in terms of the quantitative sugar excretion.

March 4 to 10, the carbohydrate was reduced to 75 gm., while fat was increased so as to keep the total calories unchanged at 1140. Glycosuria cleared up completely with the same dosage of insulin, illustrating the

fact that fat has less glycosuric effect, calory for calory, than carbohydrate. Beginning March 11, the carbohydrate was again raised to 150 gm., keeping the total calories unchanged, and glycosuria promptly returned and increased to 56 gm. by March 19.

#### REMARKS ON TABLE 16.

The diet was 50 gm. protein, 5 gm. carbohydrate and 1200 calories from August 10 to Sept. 4. Beginning August 20, the insulin dosage was 2 units per day. Glycosuria began Sept. 3 and continued to Sept. 5.

Sept. 5-30, fat was reduced by 100 gm. and carbohydrate increased by 10 gm., so as to keep the theoretical glucose value of the diet unchanged while reducing the total calories to 340. No allowance was made for the amounts of body fat and protein burned during this undernutrition period. The traces of glycosuria increased to quantitative amounts on Sept. 6 and 7, as frequently happens with changes of this kind, but by Sept. 9 the sugar had disappeared. The plasma sugar fell to an approximately normal level of 0.126 per cent. on Sept. 29.

October 1-11, the opposite change was made by giving a carbohydrate-free diet of 50 gm. protein, 150 gm. fat, and 1550 calories. The plasma sugar rose rather rapidly and glycosuria appeared on Oct. 11.

Next, a diet of 75 gm. protein, 5 gm. fat, and 345 calories was tried until Nov. 18. The purpose was to test the influence of protein by giving most of the calories in this form. An effect was evident in the continuance of traces of glycosuria until Oct. 23. The urine thereafter was sugar-free, and the plasma sugar gradually fell to a minimum of 0.139 per cent. on Nov. 16. Here again no allowance was made for the quantities of body fat consumed on the undernutrition regime.

November 19-December 2, 100 gm. of fat was added to the diet, which thus with 1245 calories became approximately a "basal" ration. It seems probable that at least 100 gm. of body fat had been burned daily in the preceding undernutrition period. Nevertheless, the fat feeding showed the usual influence. Glycosuria began Nov. 21 and continued to the end of the period. December 3-16, another 50 gm. of fat was added, raising the total calories to 1695. A decided rise of glycosuria resulted, notwithstanding increase of insulin dosage to 4 units beginning Dec. 7.

December 17-January 13, a diet of 70 gm. protein, 30 gm. fat, and 12 gm. carbohydrate was given. The theoretical glucose value was thus kept practically unchanged, while total calories were reduced to 600. Glycosuria diminished and was absent after Jan. 8, and the plasma sugar fell as low as 0.166 per cent.

January 14-20, changes of fat and protein made up a total ration of 900 calories, with no change in the chemical findings except that the falling tendency of the plasma sugar was replaced by a slight rise. January 21-28, an increase of fat made a total diet of 70 gm. protein, 12 gm. carbohydrate and 1800 calories. Glycosuria appeared on the first day and continued throughout this period.

TABLE 16  
Case No. 54

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Aug. 17	50	108	5	1200	0	.....	182 214† 192* 200	..	1
18	"	"	"	"	0	.....	199† 195* 214	..	1
19	"	"	"	"	0	.....	234† 200*	..	
20	"	"	"	"	0	.....	189 242	70	2
21	"	"	"	"	0	7.17	255† 226*	..	2
22	"	"	"	"	0	6.66	...	67	2
23	"	"	"	"	0	7.14	...	..	2
24	"	"	"	"	0	10.97	...	69	2
25	"	"	"	"	0	6.47	164	..	2
26	"	"	"	"	0	7.21	...	67	2
27	"	"	"	"	0	7.45	230†	..	2
28	"	"	"	"	0	7.13	...	69	2
29	"	"	"	"	0	7.49	187*	..	2
30	"	"	"	"	0	7.69	203	69	0
31	"	"	"	"	0	7.90	234 300*	..	0
Sept. 1	"	"	"	"	0	6.89	312	69	2
2	"	"	"	"	0	8.29	214*	..	2
3	"	"	"	"	+	8.30	272	71	2
4	"	"	"	"	+	7.71	246	..	2
5	50	8	15	340	+	9.17	288 294*	72	2
6	"	"	"	"	1.08	9.20	375*	..	2
7	"	"	"	"	2.70	8.71	366	68	2
8	"	"	"	"	+	9.11	...	..	2
9	"	"	"	"	0	9.55	...	72	2
10	"	"	"	"	0	5.96	234 300*	..	2
11	"	"	"	"	0	5.39	117 242*	70	2
12	"	"	"	"	0	9.65	200	..	2
13	"	"	"	"	0	9.37	200*	71	2
14	"	"	"	"	0	9.80	...	..	2
15	"	"	"	"	0	...	...	71	2
16	"	"	"	"	0	9.76	...	..	2
17	"	"	"	"	0	5.79	242*	70	2
18	"	"	"	"	0	8.50	195*	..	2
19	"	"	"	"	0	9.08	...	70	2
20	"	"	"	"	0	9.34	184*	..	2
21	"	"	"	"	0	8.18	154	70	2
22	"	"	"	"	0	3.28	...	..	2
23	"	"	"	"	0	9.35	117*	70	2
24	"	"	"	"	0	2.33	114	..	2
25	"	"	"	"	0	10.36	206*	69	2
26	"	"	"	"	0	9.62	164	..	2

TABLE 16.—*Continued*

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Sept.									
27	50	8	15	340	0	10.11	..	..	2
28	"	"	"	"	0	8.77	168*	..	2
29	"	"	"	"	0	8.90	126	..	3
30	"	"	"	"	0	9.20	...	..	2
Oct.									
1	50	150	0	1550	0	8.89	156	68	2
2	"	"	"	"	0	8.57	...	..	2
3	"	"	"	"	0	7.73	...	69	2
4	"	"	"	"	0	7.52	...	..	2
5	"	"	"	"	0	6.72	...	70	2
6	"	"	"	"	0	8.05	230	..	2
7	"	"	"	"	0	8.95	...	68	2
8	"	"	"	"	0	7.63	...	..	2
9	"	"	"	"	0	8.48	...	69	2
10	"	"	"	"	0	6.95	270	..	2
11	"	"	"	"	+	6.96	375*	69	2
12	75	5	0	345	+	10.50	334	..	2
13	"	"	"	"	+	10.12	...	68	2
14	"	"	"	"	+	9.83	334*	..	2
15	"	"	"	"	+	14.69	357	66	2
16	"	"	"	"	+	12.01	...	..	2
17	"	"	"	"	+	13.08	...	68	2
18	"	"	"	"	+	12.36	...	..	2
19	"	"	"	"	+	12.36	341	69	2
20	"	"	"	"	+	12.45	...	..	2
21	"	"	"	"	+	13.85	...	68	2
22	"	"	"	"	+	11.35	312	..	2
23	"	"	"	"	+	12.54	...	69	2
24	"	"	"	"	0	12.69	...	..	2
25	"	"	"	"	0	13.45	...	68	2
26	"	"	"	"	0	12.24	288*	..	2
27	"	"	"	"	0	13.36	238	69	2
28	"	"	"	"	0	12.68	...	..	2
29	"	"	"	"	0	7.60	...	..	2
30	"	"	"	"	0	13.55	206	..	2
31	"	"	"	"	0	11.72	...	70	2
Nov.									
1	"	"	"	"	0	12.04	...	..	2
2	"	"	"	"	0	13.40	...	69	2
3	"	"	"	"	0	12.41	230	..	2
4	"	"	"	"	0	12.62	...	69	2
5	"	"	"	"	0	12.24	...	..	2
6	"	"	"	"	0	13.50	...	69	2
7	"	"	"	"	0	12.24	159	..	2
8	"	"	"	"	0	12.67	...	..	2
9	"	"	"	"	0	11.75	157	..	2
10	"	"	"	"	0	13.66	...	..	2
11	"	"	"	"	0	13.39	...	..	2
12	"	"	"	"	0	15.34	...	68	2
13	"	"	"	"	0	16.97	175	..	2
14	"	"	"	"	0	12.18	...	67	2
15	"	"	"	"	0	14.94	142*	..	2
16	"	"	"	"	0	8.83	139	68	2
17	"	"	"	"	0	11.05	...	..	2
18	"	"	"	"	0	10.16	180*	..	2

TABLE 16.—Continued

Date 1922	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Nov.									
19	75	105	0	1245	0	12.92	166	67	2
20	"	"	"	"	0	10.52	...	..	2
21	"	"	"	"	+	8.36	...	70	2
22	"	"	"	"	+	6.33	...	..	2
23	"	"	"	"	9.01	7.63	...	69	2
24	"	"	"	"	5.56	11.70	...	..	2
25	"	"	"	"	++	8.92	...	67	2
26	"	"	"	"	++	10.26	366	67	2
27	"	"	"	"	8.77	11.76	...	..	2
28	"	"	"	"	8.87	12.62	...	69	2
29	"	"	"	"	17.22	10.10	...	..	2
30	"	"	"	"	9.75	9.86	...	69	2
Dec.									
1	"	"	"	"	8.81	8.81	375	..	2
2	"	"	"	"	6.87	10.30	...	68	2
3	75	155	0	1695	13.70	8.68	...	..	2
4	"	"	"	"	19.43	8.56	...	..	2
5	"	"	"	"	30.97	10.10	...	..	2
6	"	"	"	"	31.32	10.00	341	..	2
7	"	"	"	"	29.50	10.14	...	..	4
8	"	"	"	"	31.60	11.08	...	68	4
9	"	"	"	"	27.85	10.63	...	..	4
10	"	"	"	"	35.71	12.91	...	68	4
11	"	"	"	"	20.46	10.45	...	..	4
12	"	"	"	"	35.64	8.29	416	66	4
13	"	"	"	"	36.18	11.28	...	..	4
14	"	"	"	"	56.24	8.60	...	67	4
15	"	"	"	"	35.88	11.59	385	..	4
16	"	"	"	"	++++	11.33	...	69	4
17	70	30	12	600	34.32	7.94	...	..	4
18	"	"	"	"	36.26	11.30	...	70	4
19	"	"	"	"	28.70	11.88	417	..	4
20	"	"	"	"	32.38	14.25	...	69	4
21	"	"	"	"	20.44	11.43	...	..	3
22	"	"	"	"	33.02	12.90	...	71	4
23	"	"	"	"	+++	10.67	334	..	4
24	"	"	"	"	18.74	...	375	72	4
25	"	"	"	"	4.62	6.39	...	..	4
26	"	"	"	"	11.70	12.28	...	70	4
27	"	"	"	"	8.26	12.28	...	..	4
28	"	"	"	"	9.85	11.55	...	72	4
29	"	"	"	"	7.38	11.75	319	..	4
30	"	"	"	"	12.76	12.49	...	69	4
31	"	"	"	"	++++	13.75	...	..	4
1923									
Jan.									
1	"	"	"	"	7.78	12.49	...	70	4
2	"	"	"	"	12.93	...	...	..	4
3	"	"	"	"	9.23	10.79	306	69	4
4	"	"	"	"	3.91	12.66	...	..	4
5	"	"	"	"	4.74	14.64	...	69	4
6	"	"	"	"	+	...	...	..	4
7	"	"	"	"	+	11.83	226	67	4
8	"	"	"	"	+	10.75	...	..	4

TABLE 16.—Continued

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Jan.									
9	70	30	12	600	0	12.50	...	67	4
10	"	"	"	"	0	10.37	...	...	4
11	"	"	"	"	0	13.44	166	69	4
12	"	"	"	"	0	14.13	...	...	4
13	"	"	"	"	0	11.24	...	69	4
14	70	63	12	900	0	11.49	183	...	4
15	"	"	"	"	0	10.49	...	68	4
16	"	"	"	"	0	12.74	...	...	4
17	"	"	"	"	0	11.30	...	68	4
18	40	76	12	900	0	8.29	182	...	4
19	"	"	"	"	0	7.48	...	69	4
20	"	"	"	"	0	7.01	...	...	4
21	70	163	12	1800	3.52	10.39	257	68	4
22	"	"	"	"	+	9.21	...	...	4
23	"	"	"	"	17.22	8.58	319	69	4
24	"	"	"	"	14.63	9.05	...	...	4
25	"	"	"	"	10.64	9.41	...	70	4
26	"	"	"	"	20.24	10.47	306	...	4
27	"	"	"	"	25.48	11.72	...	69	4
28	"	"	"	"	18.86	12.42	...	...	4
29	100	150	12	1800	25.42	9.64	300	67	4
30	"	"	"	"	+++	4.62	...	...	4
31	"	"	"	"	58.58	12.23	...	68	4
Feb.									
1	"	"	"	"	43.74	25.32	357	...	9
2	"	"	"	"	27.30	14.57	...	66	9
3	"	"	"	"	0	11.25	...	...	9
4	"	"	"	"	9.50	14.00	346	70	12
5	"	"	"	"	7.04	7.45	...	...	12
6	"	"	"	"	26.88	10.13	...	70	12
7	"	"	"	"	9.00	13.33	294	...	12
8	"	"	"	"	6.32	14.47	...	73	15
9	"	"	"	"	2.40	14.78	...	...	15
10	"	"	"	"	3.22	14.63	288	71	15
11	"	"	"	"	+	19.08	242	...	15
12	"	"	"	"	+	16.56	220	72	15
13	"	"	"	"	+	16.67	...	...	15
14	"	"	"	"	24.24	15.59	226	72	15
15	"	"	"	"	...	...	341	...	15
16	"	"	"	"	17.01	...	300	74	15
17	"	"	"	"	41.65	...	...	...	15
18	"	"	"	"	53.62	25.11	...	73	15
19	"	"	"	"	41.60	13.05	326	...	15
20	"	"	"	"	19.45	15.01	...	72	15
21	100	150	112	2200	66.50	15.05	...	...	15
22	"	"	"	"	53.60	14.17	375	72	15
23	"	"	"	"	77.01	15.12	...	...	15
24	"	"	"	"	32.64	6.41	93*	73	15
25	"	"	"	"	40.47	13.73	...	...	20
26	"	"	"	"	27.75	11.27	331	74	20
27	"	"	"	"	68.40	11.56	...	...	20
28	"	"	"	"	72.15	12.07	...	73	20
Mar.									
1	"	"	"	"	61.44	13.59	405	...	20
2	"	"	"	"	54.60	13.87	...	73	20



TABLE 16.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Urinary Nitrogen Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.					
Mar.									
3	100	150	112	2200	59.57	15.24	...	..	20
4	"	"	"	"	66.60	14.59	...	74	30
5	"	"	"	"	43.23	10.14	400	..	30
6	"	"	"	"	51.87	13.41	...	75	30
7	"	"	"	"	64.64	15.14	429	..	30
8	"	"	"	"	60.00	13.68	...	75	36
9	"	"	"	"	37.60	14.78	...	..	36
10	"	"	"	"	20.18	13.60	...	75	36
11	"	"	"	"	14.02	10.90	...	..	44
12	150	20	112	1230	0	17.86	133	79	44
13	"	"	"	"	0	19.20	...	..	44
14	"	"	"	"	0	19.82	119	79	44
15	300	20	112	1830	0	30.28	97	..	44
16	"	"	"	"	8.35	36.15	...	80	44
17	258	20	112	1660	0	26.50	...	..	44
18	268	20	112	1700	3.68	36.12	...	81	44
19	290	20	112	1790	0	32.90	136	..	44
20	255	20	112	1650	4.45	33.20	...	81	44
21	255	20	112	1650	+	34.02	116	..	27
22	246	20	112	1600	3.14	35.80	246	82	36
23	246	20	112	1600	3.08	34.50	...	..	36
24	281	20	112	1720	0	35.85	...	82	36
25	229	20	112	1550	0	33.78	...	..	36
26	253	20	112	1640	6.20	33.70	124	82	36
27	263	20	112	1680	4.70	29.00	...	..	36
28	292	20	112	1800	4.90	36.40	...	81	30
29	272	20	112	1720	0	34.80	...	..	30
30	304	20	112	1850	15.70	38.30	107	..	30
31	282	20	112	1760	9.60	44.70	...	84	30
April									
1	257	20	112	1650	13.50	41.30	...	..	30
2	301	20	112	1833	0	39.10	131	86	30
3	282	20	112	1758	0	31.70	...	..	30
4	284	20	112	1766	0	39.20	...	..	30
5	306	20	112	1856	0	32.60	157	84	30
6	288	20	112	1783	0	38.30	...	..	30
7	276	20	112	1734	0	38.30	122	85	30
8	267	20	112	1700	0	33.00	...	..	30
9	279	20	112	1750	0	36.70	91	..	30
10	267	20	112	1700	0	35.00	...	..	30

†Blood sample taken at 3 p.m.

\*Blood sample taken at 8 p.m.

Other blood samples taken before breakfast.

January 29–February 20, the protein was increased to 100 gm., while the fat was reduced so as to keep the total calories at 1800. A decided increase of glycosuria seemed to be present on Jan. 31, as though the glycosuric effect of the protein was greater than that of the caloric equivalent of fat. The insulin dosage was then increased. With 15 units per day, the glycosuria fell to traces on Feb. 11 to 13, but rose markedly

thereafter, probably because the diet was producing an increase of body weight.

February 21–March 11, 100 gm. of carbohydrate was added to the diet without change of the protein or fat, so that the total calories were increased to 2200. The glycosuria rose, but by no means to an extent equal to the increase of carbohydrate. Beginning Feb. 25, the insulin dosage was increased, but at the close of the period on March 11 there was still glycosuria of 14 gm. with insulin dosage of 44 units. A large part of this increased requirement must certainly be attributed to the increase of total calories and body weight.

As evidence for this last statement, on March 12 the protein was increased to 150 gm., the carbohydrate was left unchanged, and the fat was reduced sharply to 20 gm., thus lowering the total calories to 1230. Glycosuria stopped immediately, and by March 14 the plasma sugar was normal. From that time until April 11, the patient ate as much protein as she was able every day. Nevertheless, on the low calory diet resulting from the reduction of fat, it became possible to keep glycosuria absent while reducing the insulin dosage to 30 units per day. This decrease of dosage was in fact made compulsory by the occurrence of occasional slight hypoglycemic reactions. Any particularly harmful influence of protein, due either to its specific dynamic action or to any hypothetical toxic or irritant effect, seems thus to be thoroughly excluded. The influence of total calories, as represented particularly in the fat ration, is demonstrated in the usual manner.

#### REMARKS ON TABLE 17.

This patient began insulin treatment on Nov. 4 with normal plasma sugar on his regular diet of 50 gm. protein, 10 gm. carbohydrate and 1100 calories, which was the limit of his tolerance as established by observations through three years of continuous residence in the Institute. Nov. 7, the diet was raised to 60 gm. protein, 20 gm. carbohydrate and 1300 calories. The reality of the limit of tolerance mentioned is shown by the fact that this small increase caused first hyperglycemia and then glycosuria, notwithstanding an increase of insulin to 6 units daily. In this case, therefore, it may be reckoned that an increase of diet by 10 gm. protein, 10 gm. carbohydrate and 14 gm. fat (200 calories) created a demand for somewhat more than 6 extra units of insulin. The pancreatic overstrain involved by slight excesses of diet, and the tendency to progressive breaking down of tolerance unless the dietary precautions are extremely strict, are thus clearly demonstrated, and are in complete harmony with the entire history of this patient, who had progressed downward very seriously during four years of slightly excessive diet and had been kept free from progressive tendencies during the three following years in the Institute.

December 17, the carbohydrate was increased to 25 gm., the protein kept at 60 gm., and the fat reduced so as to reduce the total calories to 1100. Glycosuria ceased, and the plasma sugar fell gradually to 0.130 per cent. on Jan. 8. The influence of a comparatively small change in the fat ration is here plainly perceptible.

TABLE 17  
Case No. 85

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Nov.								
4	50	95	10	1100	0	108	104	1½
5	"	"	"	"	0	...	...	1
6	"	"	"	"	0	...	103	1
7	60	109	20	1300	0	108	...	1
8	"	"	"	"	0	...	103	1
9	"	"	"	"	0	139	...	1
10	"	"	"	"	0	...	103	1
11	"	"	"	"	0	...	...	1
12	"	"	"	"	0	129	103	1
13-14	"	"	"	"	0	...	103	1
15	"	"	"	"	0	138	...	1
16	"	"	"	"	0	...	101	1
17-18	"	"	"	"	0	173	...	1
19	"	"	"	"	0	169	100	2
20-21	"	"	"	"	0	...	102	2
22	"	"	"	"	0	...	...	1
23	"	"	"	"	0	268	100	4
24-25	"	"	"	"	0	...	100	4
26-27	"	"	"	"	0	312	100	4
28	"	"	"	"	+	...	100	2
29	"	"	"	"	+	...	...	2
30	"	"	"	"	+	...	100	4
Dec.								
1	"	"	"	"	+	...	...	4
2	"	"	"	"	+	288	101	4
3-4-5	"	"	"	"	0	...	100	4
6	"	"	"	"	0	290	100	2
7	"	"	"	"	0	...	...	4
8	"	"	"	"	0	...	100	4
9	"	"	"	"	0	...	...	4
10	"	"	"	"	0	...	100	4
11	"	"	"	"	+	306	...	4
12	"	"	"	"	+	...	100	6
13-14	"	"	"	"	0	...	102	6
15	"	"	"	"	+	293	...	6
16	"	"	"	"	+	...	102	6
17-18	60	84	25	1100	+	...	103	6
19	"	"	"	"	0	...	...	6
20	"	"	"	"	0	246	102	6
21	"	"	"	"	0	...	...	4
22	"	"	"	"	0	...	102	6
23	"	"	"	"	0	192	...	6
24	"	"	"	"	0	...	102	6
25-26	"	"	"	"	0	...	102	6
27	"	"	"	"	0	145	...	6
28-29	"	"	"	"	0	...	103	6
30	"	"	"	"	0	...	104	6
31	"	"	"	"	0	160	...	6
1923								
Jan.								
1-2	"	"	"	"	...	...	104	6
3-4	"	"	"	"	0	...	102	6

TABLE 17.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Jan.								
5	60	84	25	1100	0	439	104	6
6	"	"	"	"	0	...	...	6
7	"	"	"	"	0	...	104	6
8	60	108	20	1300	0	130	...	6
9-10-11	"	"	"	"	0	...	105	6
12	"	"	"	"	0	166	...	6
13	"	"	"	"	0	...	105	6
14-20	"	"	"	"	0	182	106	8
21	60	186	20	2000	0	192	107	8
22-23	"	"	"	"	0	...	107	8
24	"	"	"	"	0	254	...	8
25	"	"	"	"	0	...	107	8
26	"	"	"	"	0	220	...	8
27	"	"	"	"	0	...	106	8
28	"	"	"	"	0	...	...	8
29	"	"	"	"	+	...	106	8
30	"	"	"	"	++	395	...	8
31	"	"	"	"	+	...	105	8
Feb.								
1	"	"	"	"	+	...	...	8
2	"	"	"	"	+	...	...	8
3	"	"	"	"	+	272	104	8
4	"	"	"	"	++	...	...	8
5	"	"	"	"	0	...	105	12
6	"	"	"	"	0	294	...	12
7	"	"	"	"	0	...	108	12
8	"	"	"	"	0	...	...	12
9	"	"	"	"	0	...	107	15
10	"	"	"	"	0	...	...	15
11	"	"	"	"	0	60	107	15
12	"	"	"	"	0	...	...	15
13	"	"	"	"	0	152	108	15
14	"	"	"	"	0	...	...	15
15	"	"	"	"	0	...	105	15
16	"	"	"	"	0	...	...	15
17	60	151	100	2000	0	182	107	15
18	"	"	"	"	0	...	...	15
19	"	"	"	"	+	...	107	15
20	"	"	"	"	8.23	334	...	15
21	"	"	"	"	20.10	...	...	15
22	"	"	"	"	53.04	...	...	15
23	"	"	"	"	32.19	448	107	15
24	"	"	"	"	52.48	...	...	15
25	"	"	"	"	37.60	...	105	15
26	"	"	"	"	75.84	...	...	21
27	"	"	"	"	62.73	319	104	21
28	"	"	"	"	29.75	...	...	21
Mar.								
1	"	"	"	"	7.00	...	104	21
2	"	"	"	"	59.60	405	...	24
3	"	"	"	"	41.60	...	104	24
4	"	"	"	"	50.05	...	...	24
5	"	"	"	"	49.70	370	105	27
6	"	"	"	"	40.40	...	...	27
7	"	"	"	"	78.71	...	103	27
					43.20	469	...	27

TABLE 17.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Mar.								
8	60	151	100	2000	21.09	...	105	33
9	"	"	"	"	14.80	429	...	33
10	"	"	"	"	13.50	...	105	33
11	"	"	"	"	+	395	...	40
12	"	"	"	"	+	...	108	40
13	"	"	"	"	0	...	...	40
14	"	"	"	"	+	...	110	40
15	"	"	"	"	0	306	...	40
16	"	"	"	"	0	...	109	40
17	"	"	"	"	0	...	...	40
18	"	"	"	"	0	...	111	40
19	60	262	100	3000	0	...	...	40
20	"	"	"	"	0	...	112	40
21	"	"	"	"	0	294	...	40
22	"	"	"	"	0	...	113	40
23	"	"	"	"	0	252	...	40
24	"	"	"	"	0	...	113	40
25	"	"	"	"	0	...	...	40
26	"	"	"	"	0	246	114	40
27	"	"	"	"	0	...	...	40
28	"	"	"	"	0	...	113	40
29	"	"	"	"	0	223	...	40
30	"	"	"	"	0	...	113	40
31	"	"	"	"	0	234	...	40
April								
1	60	362	90	3860	0	...	114	40
2	"	"	"	"	0	160	...	40
3	"	"	"	"	0	...	114	40
4	"	"	"	"	3.60	...	...	30
5	"	"	"	"	0	306	115	30
6	"	"	"	"	0	...	...	30
7	"	"	"	"	0	300	116	30
8	"	"	"	"	+	...	...	30
9	"	"	"	"	11.70	...	116	30
10	"	"	"	"	7.50	...	...	30
11	"	"	"	"	13.80	...	116	30
12	"	"	"	"	6.50	366	...	33
13	"	"	"	"	0	...	116	33
14	"	"	"	"	0	...	...	33
15	"	"	"	"	0	...	117	33
16	"	"	"	"	0	288	...	33
17	"	"	"	"	0	...	118	33
18	"	"	"	"	0	200	...	33
19	"	"	"	"	0	300	119	33
20	"	"	"	"	0	...	...	33
21	"	"	"	"	0	...	120	33
22	"	"	"	"	0	...	...	33
23	"	"	"	"	0	272	120	33
24	"	"	"	"	0	...	...	33
25	"	"	"	"	0	...	121	33
26	"	"	"	"	0	...	...	33
27	"	"	"	"	0	246	122	33
28	"	"	"	"	0	...	...	33
29	"	"	"	"	0	...	124	33
30	"	"	"	"	0	203	...	33

TABLE 17.—*Continued*

Date 1923	DIET				Glyco- suaia Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
May								
1	60	362	90	3860	0	...	125	33
2	"	"	"	"	2.3	...	...	33
3	"	"	"	"	9.0	326	125	33
4	"	"	"	"	21.5	...	...	33
5	"	"	"	"	27.2	469	124	33
6	"	"	"	"	9.9	...	...	36
7	"	"	"	"	28.4	...	124	36
8	"	"	"	"	13.2	...	...	36
9	"	"	"	"	30.1	652	125	40
10	"	"	"	"	12.2	...	...	40
11	"	"	"	"	31.2	...	125	44
12	"	"	"	"	20.2	...	...	44
13	"	"	"	"	23.1	...	126	48
14	"	"	"	"	24.8	416	...	48
15	"	"	"	"	20.0	...	127	48
16	"	"	"	"	21.8	...	...	52
17	"	"	"	"	22.2	...	128	52
18	"	"	"	"	34.1	429	...	54
19	"	"	"	"	19.6	...	129	54
20	"	"	"	"	31.7	...	...	60
21	"	"	"	"	36.3	...	130	60
22	"	"	"	"	47.7	...	...	60
23	"	"	"	"	43.7	455	130	66
24	"	"	"	"	42.9	...	...	66
25	"	"	"	"	40.4	...	...	66
26	"	"	"	"	34.7	...	129	66
27	"	"	"	"	25.4	...	130	75
28	"	"	"	"	25.6	...	...	75
29	"	"	"	"	15.0	357	131	75
30	"	"	"	"	...	...	...	80
31	"	"	"	"	10.9	...	131	80
June								
1	"	"	"	"	26.5	326	...	80
2	"	"	"	"	29.7	...	...	80

January 8, the opposite change was made, restoring the former diet of 60 gm. protein, 20 gm. carbohydrate and 1300 calories. Hyperglycemia resulted, notwithstanding an increase of insulin to 8 units per day. Jan. 21, the fat was still further increased, so as to make 2000 total calories. Glycosuria began Jan. 29, and was halted only by an increase of insulin to 12 units on Feb. 4. An increase to 15 units, beginning Feb. 8, failed to restore the original normal level of plasma sugar, hyperglycemia of 0.182 per cent. being still present on the morning of Feb. 16. It can here be calculated that an increase of diet from 1100 calories to 2000 calories, chiefly by addition of fat, created an extra requirement for at least 15 units of insulin. This requirement would probably have grown still greater had further time been allowed for the fat to manifest its full influence. The fallacy of high fat diets is thus plainly illustrated.



Feb. 16, the carbohydrate was increased to 100 gm., the protein kept unchanged at 60 gm., and the fat reduced so as to keep the total calories at 2000. Glycosuria began within two days, and reached high values. It was halted only by an increase of insulin to 40 units per day.

March 19, an increase of fat, raising the total calories to 3000, had no apparent effect whatever, and the hyperglycemia actually diminished somewhat. April 1, a further increase of fat by 100 gm. (with reduction of 10 gm. carbohydrate to maintain the same theoretical glucose value) raised the total calories to 3860. On the following day there was a particularly marked fall of plasma sugar, as though fat had largely replaced glucose in the metabolism. Furthermore, on the evenings of April 2 and 3, hypoglycemic symptoms appeared, which made it necessary to reduce the insulin dosage to 30 units, beginning April 4. On account of a return of glycosuria, it became necessary to increase the dosage to 33 units beginning April 12.

This case offers two apparent contradictions which are instructive, namely, the manifest influence of comparatively small quantities of fat in causing glycosuria and raising the insulin requirement in the earlier period, and the seeming absence of such an influence with maximum quantities of fat in the later period. The influence in question was easily manifest in the earlier period because the patient, with his low insulin dosage, was still in a state of severe diabetes. In the later period, the high insulin dosage turned the case to all intents and purposes into one of mild diabetes, and it is thus to be anticipated that larger quantities of fat and a longer period of time should be necessary for the effects to become manifest.

The instructiveness extends still further. On the face of the results, it appears that the increase of carbohydrate to 100 gm. on Feb. 16, without change in the total calories, created a very great increase of insulin requirement, namely, from 15 units to 40 units per day; also, that the subsequent great increase of fat actually reduced the tendency to hyperglycemia. Unfortunately, the experiment may not be complete for some months yet. The expectation seems justified that as the body weight rises with the high fat ration, the insulin requirement will gradually rise, not merely to 40 units but well above this dosage. It remains probable that carbohydrate, calory for calory, creates a somewhat greater need for insulin than does fat, but the difference is by no means so great as might be supposed from brief and superficial tests. The experiment is opposed to the idea that fat exerts its influence through direct conversion of any of its components into glucose.

The preceding paragraphs were written when the record extended only to April 18. It has since become possible to extend the table to June 2. The above predictions concerning the slow influence of fat are amply confirmed by the glycosuria still present with the huge dosage of 80 units. The actual requirement is probably not far from 100 units.

TABLE 18  
Case No. 839

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Nov.								
6	40	68	5	800	0	166	86	1
7	50	84	10	1000	0	175	...	1
8	"	"	"	"	0	...	...	1
9	"	"	"	"	0	...	...	1
10	"	"	"	"	0	211	...	1
11	"	"	"	"	0	...	...	1
12	"	"	"	"	0	...	...	1
13	"	"	"	"	0	223	...	2
14	"	"	"	"	0	...	...	2
15	"	"	"	"	0	...	...	2
16	"	"	"	"	0	214	...	5
17	"	"	"	"	0	289	...	5
18	"	"	"	"	0	...	...	5
19	"	"	"	"	0	...	...	5
20	"	"	"	"	0	...	...	5
21	"	"	"	"	0	...	...	5
22	"	"	"	"	0	...	...	5
23	50	140	10	1500	+	326	86	5
24	"	"	"	"	+	...	...	5
25	"	"	"	"	0	288	...	5
26	"	"	"	"	0	...	...	5
27	"	"	"	"	0	...	...	5
28	"	"	"	"	0	300	...	5
29	"	"	"	"	+	...	...	5
30	"	"	"	"	+++	...	...	5
Dec.								
1	"	"	"	"	+	319	...	5
2	"	"	"	"	+++	...	...	5
3	"	"	"	"	4.09	...	...	5
4	"	"	"	"	9.60	...	...	5
5	"	"	"	"	18.31	...	...	2½
6	"	"	"	"	18.33	469	...	2½
7	"	"	"	"	31.26	...	...	5
8	"	"	"	"	7.45	500	...	9
9	"	"	"	"	14.14	...	...	9
10	"	"	"	"	36.48	...	...	0
11	"	"	"	"	13.55	...	...	6
12	"	"	"	"	51.41	...	...	9
13	"	"	"	"	43.18	...	...	9
14	"	"	"	"	30.68	...	...	9
15	"	"	"	"	28.73	455	...	9
16	"	"	"	"	++++	...	...	9
17	50	40	20	640	12.77	...	89	9
18	"	"	"	"	8.79	...	...	9
19	"	"	"	"	8.71	...	...	9
20	"	"	"	"	12.49	441	...	9
21	"	"	"	"	14.50	...	...	6
22	"	"	"	"	38.38	416	...	9
23	"	"	"	"	24.58	...	...	9
24	"	"	"	"	31.75	441	...	9
25	"	"	"	"	26.78	...	...	9
26	"	"	"	"	47.60	...	...	9
27	"	"	"	"	...	...	...	9

TABLE 18.—*Continued*

Date 1922	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Dec.								
28	50	40	20	640	17.34	441	...	9
29	"	"	"	"	33.48	...	...	9
30	"	"	"	"	66.99	...	...	9
31	"	"	"	"	66.40	...	...	9
1923								
Jan.								
1	"	"	"	"	81.56	...	...	9
2	"	"	"	"	39.06	...	...	9
3	"	"	"	"	53.82	518	...	9
4	"	"	"	"	38.87	...	...	9
5	"	"	"	"	49.20	...	88	9
6	"	"	"	"	19.29	...	...	9
7	"	"	"	"	30.07	...	...	12
8	"	"	"	"	27.01	...	...	12
9	"	"	"	"	36.25	455	...	12
10	"	"	"	"	36.05	...	...	12
11	"	"	"	"	57.96	...	...	12
12	"	"	"	"	61.80	...	...	12
13	"	"	"	"	54.00	...	...	12
14	"	"	"	"	48.96	...	...	15
15	"	"	"	"	15.80	...	...	15
16	"	"	"	"	51.45	...	...	15
17	"	"	"	"	45.20	484	...	15
18	"	"	"	"	48.20	...	...	21
19	"	"	"	"	46.20	...	...	21
20	"	"	"	"	18.25	...	...	21
21	"	"	"	"	48.60	395	...	21
22	"	"	"	"	41.10	...	...	24
23	"	"	"	"	36.24	...	...	24
24	"	"	"	"	12.24	416	...	24
25	"	"	"	"	7.13	...	...	24
26	"	"	"	"	3.99	385	...	24
27	"	"	"	"	12.72	...	...	24
28	"	"	"	"	6.51	349	...	24
29	"	"	"	"	+	...	...	24
30	"	"	"	"	+	334	...	24
31	"	"	"	"	0	...	...	24
Feb.								
1	50	140	10	1500	0	294	87	24
2	"	"	"	"	0	...	...	24
3	"	"	"	"	0	...	...	21
4	"	"	"	"	0	...	...	24
5	"	"	"	"	0	300	...	24
6	"	"	"	"	0	...	...	24
7	"	"	"	"	0	334	...	24
8	"	"	"	"	0	...	...	24
9	"	"	"	"	0	...	...	24
10	"	"	"	"	0	254	...	24
11	50	100	100	1500	5.35	...	90	24
12	"	"	"	"	33.12	...	...	24
13	"	"	"	"	43.89	536	...	24
14	"	"	"	"	82.25	...	...	24
15	"	"	"	"	...	...	...	24
16	"	"	"	"	56.70	...	...	27

TABLE 18.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
Feb.								
17	50	100	100	1500	41.20	455	...	27
18	"	"	"	"	44.64	...	...	30
19	"	"	"	"	0	...	...	30
20	"	"	"	"	35.09	...	...	30
21	"	"	"	"	27.90	429	...	33
22	"	"	"	"	17.04	...	...	33
23	"	"	"	"	10.92	...	...	33
24	"	"	"	"	20.40	395	...	33
25	"	"	"	"	4.76	...	...	33
26	"	"	"	"	7.75	...	...	33
27	"	"	"	"	5.46	300	...	33
28	"	"	"	"	9.86	...	...	33
Mar.								
1	"	"	"	"	7.03	...	93	33
2	"	"	"	"	16.72	344	...	33
3	"	"	"	"	17.43	395	...	33
4	"	"	"	"	45.85	375	...	33
5	"	"	"	"	7.36	385	...	33
6	"	"	"	"	...	395	...	33
7	"	"	"	"	34.80	416	97	33
8	"	"	"	"	12.30	...	...	33
9	"	"	"	"	16.45	385	...	33
10	"	"	"	"	18.30	...	...	33
11	"	"	"	"	8.64	...	...	40
12	"	"	"	"	...	...	...	40
13	"	"	"	"	6.60	357	...	40
14	"	"	"	"	7.85	...	...	40
15	"	"	"	"	18.52	...	...	40
16	"	"	"	"	14.85	...	...	40
17	"	"	"	"	2.44	375	...	40
18	"	"	"	"	6.11	...	...	40
19	"	"	"	"	8.12	...	...	40
20	"	"	"	"	24.05	...	...	40
21	"	"	"	"	4.58	385	...	42
22	"	"	"	"	8.17	...	...	42
23	"	"	"	"	3.26	...	...	42
24	"	"	"	"	10.06	306	...	42
25	60	151	100	2000	5.12	...	100	42
26	"	"	"	"	4.29	326	...	42
27	"	"	"	"	3.46	...	102	42
28	"	"	"	"	4.50	...	...	42
29	"	"	"	"	0	334	103	42
30	"	"	"	"	0	...	...	42
31	"	"	"	"	0	326	...	42
April								
1	"	"	"	"	2.80	...	...	42
2	"	"	"	"	+	...	...	42
3	"	"	"	"	2.50	319	102	42
4	"	"	"	"	6.70	...	...	42
5	"	"	"	"	3.20	...	103	45
6	"	"	"	"	5.80	416	...	45
7	"	"	"	"	1.80	...	...	45
8	"	"	"	"	5.70	...	...	48
9	"	"	"	"	+	...	...	48
10	"	"	"	"	0	...	...	48

TABLE 18.—*Continued*

Date 1923	DIET				Glyco- suria Gm.	Plasma Sugar Mg. Per 100 cc.	Weight, Lb.	Insulin, Units
	P. Gm.	F. Gm.	C.H. Gm.	Cal.				
April								
11	60	151	100	2000	2 10	...	105	48
12	"	"	"	"	+++	334	...	50
13	"	"	"	"	3.40	...	...	50
14	"	"	"	"	10.40	...	...	50
15	"	"	"	"	9.80	...	105	50
16	"	"	"	"	...	...	...	50
17	"	"	"	"	3 50	395	...	50
18	"	"	"	"	3.40	...	...	50
19	"	"	"	"	0	...	...	50
20	"	"	"	"	4 50	349	...	50
21	70	51	110	1179	4.00	...	...	50
22	"	"	"	"	0	...	...	50
23	"	"	"	"	0	...	...	50
24	"	"	"	"	+	319	...	50
25	"	"	"	"	0	...	108	50
26	"	"	"	"	0	...	...	50
27	"	"	"	"	0	...	...	50
28	"	"	"	"	0	312	...	50
29	"	"	"	"	0	...	...	50
30	"	"	"	"	0	...	110	50
May								
1	"	"	"	"	0	...	...	50
2	"	"	"	"	0	238	110	50

## REMARKS ON TABLE 18.

As mentioned in this patient's history, the rise of plasma sugar to 0.326 per cent. on the morning of Nov. 23 showed that the insulin dosage of 5 units was inadequate to balance the slight increase of diet which had been made.

Beginning Nov. 23, the fat of the diet was further increased, so as to raise the total calories to 1500. Marked and increasing glycosuria resulted, notwithstanding an increase of insulin to 9 units per day.

Dec. 17, the protein was kept unchanged, the carbohydrate was increased by 10 gm., and the fat was diminished by 100 gm., so as to keep the theoretical glucose value of the diet unchanged, while reducing the total calories to 640. It was anticipated that this undernutrition would result in clearing up the glycosuria with the same dosage of insulin. There was a promise of fulfillment of this expectation in the marked fall of glycosuria which occurred during the first two days. The heavy glycosuria, however, together with the small dosage of insulin, was evidently responsible for a rapidly progressing loss of tolerance in such a severe case. Accordingly, after Dec. 22, it is noticeable that the glycosuria rose again to high figures. In support of this view, that the experiment failed because it was wrongly planned with regard to the

existing conditions, is the fact that an increase of dosage to 24 units was necessary in order to abolish the glycosuria (Jan. 31) on this low diet.

Feb. 1, the experiment was resumed on a new plan. The diet of 50 gm. protein, 10 gm. carbohydrate and 1500 calories was restored, and during the following ten days glycosuria remained absent and the plasma sugar showed no important change. Feb. 11, the protein was left unchanged, the carbohydrate was increased to 100 gm., and fat was correspondingly reduced so as to keep the total calories constant at 1500. Glycosuria resulted immediately, and quickly became heavy. It was variable, illustrating the usual inaccuracy of the quantitative sugar excretion as a measure of tolerance, but it persisted to March 24, notwithstanding an increase of insulin dosage to 42 units per day.

March 25, an unimportant increase of protein to 60 gm. was made for the sake of comfort. The carbohydrate was left unchanged, and the fat was increased so as to raise the total calories to 2000. With this change, the glycosuria on the whole was lower than before, and the urine was actually sugar-free on several days. A superficial view would indicate that the added fat had no harmful influence whatever, and was possibly beneficial through additional sparing of protein.

It has been noted, however, that with the comparatively mild state of diabetes brought about by this high insulin dosage, the effect of fat is slow in becoming manifest. To those familiar with such experiments, the tendency to an increase of glycosuria after April 12, notwithstanding an increase of insulin to 50 units per day, suggests that the harmfulness of the fat is already in evidence. To test this idea, on April 21 the fat intake was reduced by 100 gm., while the protein was increased to 70 gm., and the carbohydrate to 110 gm. The total calories were thus considerably reduced, while the theoretical glucose value was very slightly increased. The usual effect of the lower nutrition is seen in the cessation of glycosuria and reduction of hyperglycemia.

## DISCUSSION

### 1. Physiological Role of Insulin.

The experiments with insulin confirm in all details the former observations with different diets in diabetic patients and animals. The food which tends most strongly to produce glycosuria, and which therefore breaks down the tolerance most rapidly is carbohydrate. Protein comes second, and its glycosuric action in average cases is not equal to its theoretical glucose value. Fat seems to be important chiefly through the number of calories furnished by it, rather than as a theoretical direct source of glucose. The most important factor governing the insulin requirement with any ordinary plan of diet is not the carbohydrate content but the total caloric content. Superficial investigation may give ap-



parent support to some formula for reckoning insulin dosage according to the glucose of the diet or in the urine, but all such rules are based on arbitrarily fixed experimental conditions, and therefore have no deep significance. Suitable variations of the non-glucose elements of the diet readily expose these errors. Care is also necessary not to overlook the effects of fat, which have repeatedly been described as slow and insidious. These effects are more rapid and obvious in proportion to the severity of the case, but in all grades of diabetes they have almost universally been misinterpreted as "spontaneous downward progress." They are always plainly demonstrable under accurate experimental conditions, irrespective whether the basis of judgment be hyperglycemia or glycosuria. Obviously the harmfulness of fat is not shown except when it is so used as to overtax the tolerance. Therefore small quantities show evident harm in severe cases, often within a few days, while in milder cases the quantities used must be correspondingly larger, and the experimental period must often be lengthened to many months.

These facts correspond to the known history of diabetic therapy. Limitation of carbohydrate alone was first employed. It was necessarily unsuccessful in the severest cases, because the glycosuria was not thus stopped and the high protein-fat diet entailed early death from acidosis. But in milder cases, glycosuria would be at least temporarily controlled, and downward progress would be delayed. Therefore, on the whole, the Rollo treatment was beneficial. The next step, during the period distinguished by the names of Bouchardat and Naunyn, consisted in limitation of protein as a source of sugar in the body. Furthermore, Bouchardat with his maxim "*manger le moins possible*," and Cantani and Naunyn with their fast days, introduced an element, the importance of which they failed to grasp fully in either theory or practice. Only one further dietary restriction was possible, and that was the limitation of fat and total calories. This method alone made possible the control of symptoms in the graver cases, and physicians who have not used undernutrition in proportion to the severity of the diabetes have not controlled really severe cases. The idea of such writers as Kolisch, Petrén, and Newburgh and Marsh, that protein is specifically injurious in diabetes while fat can be given freely without harm, was contrary to the facts previously established, and is completely exploded by the experiments with insulin. Newburgh and Marsh have merely illustrated

the general rule that the composition of the diet may vary widely without harm, so long as the total calories are limited. Whether or not many writers yet agree that the progressiveness of diabetic cases in general can be halted by sufficiently strict total dietary regulation, the majority are at least convinced that the progress is delayed for a longer time by this method than by any other.

Before conclusions are drawn too positively concerning a specific relation of insulin to glucose, one point should be established beyond doubt, namely, whether the apparently higher insulin requirement for the assimilation of carbohydrate as compared with other foods is real and permanent or accidental and transitory. Carbohydrate is somehow quicker in its action, and patients thus lose tolerance more rapidly, and for the time being require higher insulin dosage for assimilation of the same number of calories. But if the decline of tolerance be prevented by administration of whatever dosage of insulin is found necessary for a given diet, is it certain that at the end of a year the dosage will still be higher for carbohydrate than for the caloric equivalent of fat? A distant comparison with the relations of starch and sugar may be possible. Most clinicians believe that sugar is more dangerous to diabetics than starch, because it floods the body and overtakes the weakened tolerance more violently. Glucose has actually proved useful for breaking down the tolerance of mildly diabetic dogs which seemed able to tolerate bread diet indefinitely. Yet there is no doubt that starch and glucose are identical in their nature and in the total demand for insulin in their assimilation. Our experiments make it certain that, especially with high diets and with periods of observation extending into several months, the difference between carbohydrate and fat is relatively small. From a practical standpoint, a diet which is liberal in calories may advantageously contain 100 grams or more of carbohydrate, because it is thus so much more pleasing than an almost exclusively fat diet, and the ultimate difference in insulin requirement is not great enough to be of practical importance. The hyperglycemia from fat, though slower in onset, is also more stubborn and resistant than that from carbohydrate. The hyperglycemia from fat feeding in normal persons reported by Atkinson,<sup>16</sup> and our more marked examples in epileptics,<sup>27</sup> contribute further to raise the question whether hyperglycemia or glycosuria may not be merely an expression of a general metabolic disturbance, rather than an evidence of impairment of glucose assimilation alone.

Regardless of hypotheses of this kind, the essential fact is that the insulin requirement or consumption of the body is very greatly influenced by fat or other non-carbohydrate energy carriers (alcohol) in the diet. For the explanation of this fact only the three following assumptions are possible:

(a) It may be assumed that insulin is utilized solely in glucose assimilation, but the supply of other food materials somehow interferes with this process. If it were a simple question of cell nutrition, it might be easy to understand that a plethora of other foodstuffs might tax the general combustion and storage capacity of the cell, so that glucose might be disposed of less efficiently than if the supply of other foods were abolished or restricted. But the real question involved, namely, why the cell should require or consume a much larger quantity of insulin under these circumstances, seems altogether unanswered by this assumption. The influence of body weight upon assimilation or upon the insulin requirement seems also unexplainable on this basis.

(b) It may be assumed that fat and other foodstuffs are converted into glucose, and that the supply of fat therefore influences glycosuria or the insulin requirement in this manner. A wholesale conversion, as accepted by von Noorden,<sup>28</sup> Geelmuyden,<sup>29, 30</sup> and a surprising proportion of European writers, seems to be excluded by the well established facts concerning D:N ratios and respiratory quotients. On the other hand, Atkinson<sup>16</sup> seems to have demonstrated a limited conversion of fatty acids into sugar in normal subjects, and the process may be supposed to be augmented to a maximum by the glucose hunger of the diabetic organism. In this way fat would furnish more glucose than that reckoned by Woodyatt merely from the glycerol content, and the respiration calorimeter is not such a precise instrument that it could infallibly detect this occurrence on a limited scale. Five facts, however, require notice. First, alcohol acts much like fat in diabetes, and a conversion of any considerable quantity of alcohol into glucose seems difficult to demonstrate or accept. Second, fat feeding may markedly influence glycosuria or the insulin requirement, even when it does not greatly alter the actual quantity of fat entering into the metabolism (e. g., the attempts to use so-called "basal" diets in place of fasting). Third, the effect of fat in producing glycosuria is so much slower than that of carbohydrate that a direct conversion seems improbable. Fourth, fat apparently is unable to prevent hypoglycemia from

insulin unless the diet contains a sufficient quantity of some food that is a direct source of carbohydrate. Case 1073 (table 13) furnishes an illustration, and the same facts seem incidentally to militate against the theory that the glycerol of fat may be regarded as strictly on a par with carbohydrate in the diet. Fifth, and perhaps most important, phlorizin glycosuria is not influenced by fat feeding or by the body weight. Here the loss of sugar, equalling or exceeding that of true diabetes, does not create a glucose hunger that causes any demonstrable conversion of fat into sugar. Phlorizin poisoning actually represents the condition which Woodyatt and most other writers have supposed diabetes to be, namely, a disturbance of glucose utilization alone. A diet calculated to supply adequate calories to the organism without producing acidosis would be the ideal treatment for phlorizin poisoning. There is no possible benefit from undernutrition in phlorizin poisoning. For all the above reasons an explanation based on an assumed conversion of fat into glucose in diabetes seems improbable.

(c) A third assumption may be that insulin is directly concerned with the total metabolism, and that the special prominence of glucose in the blood and urine means only that the surplus food most readily escapes from the cells and from the body in this form, and does not prove any more intimate connection of insulin with glucose metabolism than with the metabolism of other foods. The two time-honored objections to this hypothesis are weighty but by no means conclusive. (1) From the fact that levulose and various substances closely related chemically or metabolically to glucose are either utilized by totally depancreatized dogs and severely diabetic patients, or are excreted in the urine only after conversion into glucose, it has been deduced that the assimilative defect in diabetes is limited strictly to the glucose molecule. There is evidence that the liver is the most active organ in disposing of substances such as levulose, and all cells perhaps retain some primitive power of disposing of materials which are foreign to the normal metabolism, or of abnormal quantities of the intermediary products of normal metabolism, and the method of disposal seems to consist in chemical conversions which restore the ordinary metabolic conditions. Such a power may be a phase of the defense mechanism of protoplasm, or of any of the numerous chemical processes of the body which are independent of the specialized nutritive process in



which insulin is concerned. It is conceivable that by this primordial power of protoplasm, levulose, lactic acid or anything else akin to glucose may be partly burned or partly converted into glycogen or glucose, and the specific metabolic defect then becomes evident through the fact that these latter materials are not employed for normal nutrition but are excreted in the urine. Glucose itself is perhaps sometimes disposed of by such a primordial mechanism, as the totally depancreatized dog under various circumstances may excrete far less than the theoretical quantities of glucose and yet not seem to be benefited by its retention. (2) The essential basis of the belief that insulin is connected solely with carbohydrate metabolism is doubtless the elementary fact that after pancreatectomy or in "total" human diabetes carbohydrate and the carbohydrate moiety of protein are not burned, while fat and the non-carbohydrate part of protein are burned, as shown plainly by respiratory analyses. But the very prominence of the abnormality of carbohydrate metabolism has caused nearly everybody to overlook the plain indications of a disorder of total metabolism. The asthenia and early death of the totally depancreatized dog are not adequately explained by either starvation or loss of sugar. Phlorizinized dogs may lose fully as much sugar and nitrogen, but they never display the inability to resist infection or heal wounds which is so characteristic of depancreatized dogs. There is plenty of proof that depancreatized animals burn fat and part of the protein molecule to the usual end products, but no proof whatever that the processes and the results of such combustion are normal. On the contrary, the inability of the depancreatized dog to live any long time on the non-carbohydrate materials at its disposal seems to indicate that the catabolism of such materials in the absence of insulin does not have the normal beneficial results. The anabolic role of insulin must not be forgotten, and the rapid wasting and failure of wound healing make it questionable whether totally depancreatized dogs possess the slightest remnant of ability to rebuild protoplasm. If it be conceded that insulin is concerned directly in total metabolism, it may conceivably play its part at some intermediary chemical stage, as represented for example by glyoxal<sup>31</sup>, in which all food materials perhaps come together. But the speculative nature of any such hypothesis must be plainly kept in view.

The writer is not prepared to adopt any of the three assumptions, as the evidence is too scanty and insecure for a decision.

The one fact which is positively established, and which will withstand all assaults, is that the requirement or consumption of insulin by the body is governed not merely by carbohydrate but also by fat and all other energy carriers in the diet. This fact will furnish a sound basis for investigation of the physiological role of insulin, and will guard against the costly blunders that are apt to arise from the fixed idea of most investigators, especially chemists, that the islands of Langerhans merely produce some kind of a ferment for the utilization of glucose. Anyone seeking to discover the function of insulin should undertake the task with the full realization that success means an explanation not only of the diabetic abnormality but also of the entire process of normal nutrition.

## *2. Relation of Insulin to Body Weight and Composition.*

As diabetes with obesity is comparatively mild, while extreme emaciation is reached only in the very severe stage when the power of regaining tolerance is necessarily slight, it has never been possible to form any clear judgment whether the food tolerance is increased more by reduction of the fat stores or of the active protoplasm of the body. The fact is definite, however, that at all levels of nutrition the body weight and the food tolerance of the diabetic behave like the two pans of a balance; as one goes up, the other goes down. In practice, advantage is taken of this fact to enable patients to assimilate enough food to support life. Aspersions have been cast upon the so-called "starvation treatment" because it could only maintain life in the cases of maximal severity on a basis of extreme privation and emaciation. Opponents have had nothing better to offer, however, in such cases than a policy of feeding beyond the tolerance, involving a more wretched life of active symptoms and complications and a much earlier death. Attention was previously called<sup>1</sup> to the paradox that in all ordinary cases undernutrition really leads to the best nutrition. The patient with low tolerance loses both tolerance and weight if fed beyond his tolerance; if fed constantly to the extreme verge of his tolerance he gradually starves to death; but by strict initial undernutrition he is enabled to assimilate not only a living diet but a higher diet than is possible under any other method. On the theoretical side, however abstruse or fantastic the idea may appear, these facts leave no escape from the conclusion that the total body mass somehow constitutes a load



upon the internal function of the pancreas. It is necessary to view diabetes on its anabolic as well as on the usual catabolic aspect. The place held by obesity in causing or predisposing to diabetes, as shown most clearly by Joslin,<sup>32</sup> and the influence of weight changes in treatment, indicate that as food materials are accumulated, either in the living protoplasm or in inert pabulum (glycogen, fat, possibly "reserve" protein), the pancreatic island function is somehow taxed to maintain this accumulation. Weakening of this function may result in breakdown of these materials, so that the food tolerance is minimal and glycosuria may occur even on fasting. Lightening of the anabolic load by reduction of body weight shows its effect in the catabolic function, which is more easily observed and therefore has received almost exclusive attention; and thus the same patient after reduction of weight may "tolerate" a very liberal diet of protein, carbohydrate and fat. For a fruitful study of diabetes, these anabolic and catabolic processes should be viewed not as separate phenomena but merely as the two sides of the one physiological function of insulin.

### 3. *Minimum Insulin Requirement.*

The insulin requirement can be determined fairly accurately in the totally depancreatized animal, though the poor food absorption will limit the accuracy chiefly to the fasting state or to the assimilation of parenterally injected glucose. Bliss<sup>33</sup> found the insulin dosage necessary to control glycosuria and hyperglycemia in a totally depancreatized dog to be approximately 6 units per day. The dog was extremely emaciated, and, though fed, apparently absorbed little food. This dosage is therefore perhaps nearly minimal for dogs of this size. A three-year-old girl (patient No. 918) in extreme emaciation after prolonged undernutrition had almost the same weight as this dog. On general principles it should be expected that the dog's requirement should be higher than the human, kilogram for kilogram, because of the higher metabolism. This child's tolerance was barely 100 calories, and the insulin dosage necessary to prevent glycosuria on a diet of 400 calories was about 3 units per day. It is therefore probably not far wrong to reckon that the child's pancreas was producing no more than 1 unit of insulin per day, and that the actual insulin consumption on a bare maintenance diet of 400 calories was about 4 units per day. These figures are probably minimal for a young child. The ten-year-old boy, No. 1034, had nearly "total" diabetes.

Even with allowance for a little insulin production by his pancreas, he probably consumed scarcely more than 8 units per day on his diet of 804 calories. His weight was 32 pounds, as compared with 22 pounds for the girl just mentioned. His requirement of insulin was probably nearly minimal for his size. The adult woman, No. 839, seemed to require 24 units of insulin for a diet of 50 gm. protein, 20 gm. carbohydrate and 640 calories, when her weight was 87 pounds. The figures are not minimal, however, as long as the diet and the nutritive state are anything more than minimal. On the other hand the woman, No. 1054, weighing 83 pounds, was free from glycosuria on a 300 calory diet with 8 units of insulin per day, but it is probable that her own pancreas added a few units to this supply. The best example is probably the woman, No. 2186, who, when emaciated to 78 pounds, seemed to require approximately 12 units of insulin for diets of 300 to 400 calories.

These quantities of insulin provide only for the metabolism or inanition. The figures given, and similar ones elsewhere in our records, show that any "basal" diet—i.e., any ration which will prevent loss of body tissue on even the lowest plane of nutrition—must at least double these requirements. Accordingly, the lowest daily insulin supply on which life can be indefinitely maintained is probably in the neighborhood of 6 or 8 units for very young children and 24 units for adults. It must be supposed that the pancreatic islands of emaciated diabetics who have lived a number of years on the lowest possible diets must have been capable of supplying at least these quantities of insulin.

#### *4. Quantitative Relations of Insulin to Food.*

From the first discovery of insulin, there seems to have been a widespread desire on the part of physicians and an equally widespread search by investigators for some fixed general rule, by which a certain dosage of insulin could be reckoned as equivalent to a certain quantity of food, particularly carbohydrate. It would appear that a better knowledge of diabetes might have saved much labor of this kind. In the first place, diabetic cases differ notoriously, and the tendency to gain tolerance with efficient treatment and perhaps to lose tolerance with glycosuria seems to vary among individuals even with the use of insulin. The only way to obtain results independent of the functional reaction of the patient's own pancreas might be to limit the tests to patients with

"total" diabetes. Genuine absence of pancreatic island function is a great rarity in human patients, if it ever actually exists, and past experience has proved sufficiently that many at least of the cases with high or maximal D:N ratios can be cleared up wholly or partially by sufficiently stringent undernutrition. We feel that a number of the cases which we have cleared up by extreme measures in this Institute (e.g., Nos. 918, 1054, 1016, 1065, 1212, 1286, and possibly others such as 54, 174, 839, 1194 and 1304, in paper 1) were more severe than many of those in which authors using higher diets have described the diabetes as "total." Whether this arbitrary assumption be granted or not, the fact remains that the insulin requirement even of "totally" diabetic cases is not a constant but is subject to change with times and circumstances. It is emphatically untrue that brief tests of a few days can decide the food assimilation or the insulin requirement. Even for carbohydrate, a distinction must be recognized between mere temporary combustion or storage under the stimulus of plethora, and the quantity which can actually be assimilated permanently in a true sense without glycosuria. The powerful influence of fat is generally missed in short tests, and the body weight and other influences which affect the absolute insulin requirement are necessarily operative even in "total" cases.

Leaving the discussion of these influences to the following section, we may return to the fact that "total" cases are rare, and the quest of physicians and investigators has been for rules that would apply to the great mass of ordinary cases. The experimental diet changes described in these three papers afford a number of examples of the quantitative changes of insulin necessary to create tolerance for certain quantities of foods. By definition, the tolerance is considered to be the highest quantity of food which a diabetic can assimilate for a long or indefinite time without glycosuria. Any other definition appears to be contrary to both the historical and the scientific meaning of the term. A partial selection has been made of observations from all three papers which illustrate these quantitative relations, in two ways.

(a) The first test consists in placing a patient on a certain diet for a sufficient length of time to determine the insulin requirement with fair certainty, and then to increase this diet by a certain quantity of one kind of food. Two methods are open; either to add the extra food with its calories, or to make a simultaneous subtraction of some other food (generally fat) so as to keep the

total caloric intake unchanged. The two methods are by no means equivalent, and though each has its advantages, the former one creates some confusion of the specific influence of the food with the general influence of increase of calories and perhaps of body weight. Tables 19 to 22 summarize some of these observations.

In addition to the individuality of the patient and a multitude of possible variables, questions arise concerning quantitative interrelations between the four factors here particularly concerned. Is the addition of a certain number of insulin units of equal value, whether the preceding insulin dosage has been large or small? Is it of equal value with high diets and with low diets? Does the addition of a certain quantity of food act the same with high insulin dosage as with low dosage? Does it act the same

TABLE 19  
Increase of Carbohydrate with Added Calories

Case No.	Body Wgt., Lb.	FIRST DIET				SECOND DIET				Increase of C.H. Gm.	INSULIN REQUIREMENT UNITS PER DAY			Remarks
		P. Gm.	F. Gm.	C.H. Gm.	Cal.	P. Gm.	F. Gm.	C.H. Gm.	Cal.		For First Diet	For Second Diet	Increase	
54	72	100	150	12	1800	100	150	140	2200	100	15	44	29	Higher total diet
174	66	45	64	10	800	45	64	30	880	20	2	4	2	
	69	45	64	30	880	45	60	50	920	20	4	20	16	
529	45	50	113	20	1300	50	113	100	1620	80	6	15	9	
806	40	40	32	50	650	40	32	75	750	25	3	6	3	
878	74	60	164	20	1800	60	164	50	1920	30	6	8	2	
	90	60	151	100	2000	120	151	200	2640	100	30	45	15	
1303	43	25	10	5	210	30	11	1100	659	105	4	8	4	Undernutrition
1316	113	50	62	10	800	50	62	110	1200	100	4	15	11	
1317	?	60	231	40	2479	60	231	140	2879	100	30	50	20	
2214	90	30	15	5	275	30	15	75	555	70	2	6	4	

TABLE 20  
Increase of Carbohydrate Without Changing Calories

Case No.	Body Wgt., Lb.	FIRST DIET				SECOND DIET				In-crease of C.H. Gm.	INSULIN RE-QUIREMENT UNITS PER DAY		In-crease
		P. Gm.	F. Gm.	C.H. Gm.	Cal.	P. Gm.	F. Gm.	C.H. Gm.	Cal.		For First Diet	For Second Diet	
539	40	50	72	50	1050	50	50	100	1050	50	18	24	6
574	27	60	60	20	860	60	42	60	860	40	12	15	3
783	83	60	95	25	1200	60	62	100	1200	75	6	15	9
878	80	60	177	40	2000	60	151	100	2000	60	30	30	0
1065	72	20	130	1	1254	40	103	40	1254	39	8	15	7



TABLE 21  
Increase of Protein With Calories

Case No.	Body Wgt., Lb.	FIRST DIET				SECOND DIET				In-crease of Protein Gm.	INSULIN RE-QUIREMENT UNITS PER DAY		In-crease
		P. Gm.	F. Gm.	C.H. Gm.	Cal.	P. Gm.	F. Gm.	C.H. Gm.	Cal.		For First Diet	For Second Diet	
70	130	30	95	5	995	250	95	5	1855	220	12	16	4
653	110	30	206	5	2000	200	131	5	2000	170	4	16	12
1276	104	50	108	40	1340	175	108	40	1832	75	4	15	11

TABLE 22  
Increase of Fat

Case No.	Body Wgt., Lb.	FIRST DIET				SECOND DIET				In-crease of Fat Gm.	INSULIN RE-QUIREMENT UNITS PER DAY		In-crease
		P. Gm.	F. Gm.	C.H. Gm.	Cal.	P. Gm.	F. Gm.	C.H. Gm.	Cal.		For First Diet	For Second Diet	
229	36	15	0	15	120	30	28	5	400	28	2	8	6
574	21	60	10	11	374	60	60	20	860	50	2	12	10
806	40	40	32	75	750	40	115	75	1500	83	6	15	9
823	100	55	42	50	800	55	175	50	2000	133	21	45	24
878	77	60	133	40	1600	60	177	40	200	44	12	30	18
1005	128	35	0	20	220	35	150	5	1510	150	1	6	5
1065	72	20	29	1	350	20	130	1	1254	101	2	8	6
	75	40	103	40	1254	50	126	40	1500	23	15	30	15
1073	113	40	10	24	346	40	160	24	1696	150	6	32	26
1194	105	100	10	10	530	100	117	10	1500	107	1	8	7
2214	90	100	6	100	855	80	206	80	2500	200	6	12	6

when the quantities of other foods are high as when they are low? The tables show entirely bizarre results with reference to the number of units of insulin apparently required per gram of any kind of food, and Section 5 below will suggest reasons why the above questions must generally be answered in the negative.

(b) The second plan has consisted in comparisons of the tolerance of different patients. There has been a great advantage in having patients whose assimilative power has been accurately known, frequently for several years back. It has been positively established that they could take certain diets without any symptoms or progressive tendency, and that very small in-

creases above the established diet would regularly and quickly bring on symptoms. The question has been, then, starting with patients whose tolerance in this permanent sense is known, whether the increase of tolerance with identical doses of insulin will be identical, or whether conversely the same increase of diet will produce the same increase of insulin requirement. Is it possible to measure the severity of diabetic cases rigidly in terms of insulin, affirming that the one with the highest insulin requirement on a given diet is invariably the most severe? Shall the insulin requirements be measured in absolute figures, or in units per kilogram, or in proportion to the total metabolism?

TABLE 23  
Insulin Requirements of Different Patients

Case No.	Present Age Yrs.	ORIGINAL TOLERANCE				Body Wgt. Lb.	DIET WITH INSULIN				Body Wgt. Lb.	Insulin Requirement Units per day
		P. Gm.	F. Gm.	C.H. Gm.	Cal.		P. Gm.	F. Gm.	C.H. Gm.	Cal.		
3	22	50	86	5	1000	78	80	197	100	2500	88	20
24	31	50	82	15	1000	80	60	151	100	2000	90	40
54	44	45	67	3	800	62	100	150	112	2200	79	44
85	35	55	104	10	1200	100	60	151	100	2000	108	40
191	53	60	135	10	1500	97	100	133	100	2000	95	24
783	24	55	80	15	1000	81	60	151	100	2000	87	20
839	45	40	91	5	1000	82	60	151	100	2000	105	54
878	46	55	138	10	1500	74	60	151	100	2000	82	30
989	9	50	82	15	1000	35	60	151	100	2000	60	66
1034	9	0	0	0	0	33	60	151	100	2000	40	24

Table 23 shows a group of severely diabetic patients, varying widely in body weight and other physical characters, and with tolerance sometimes nearly identical and sometimes higher or lower as compared one with the other. The final diets were not always identical, but care was taken at some time in the course of the treatment to place most of these patients on a standard test diet, namely 60 gm. protein, 100 gm. carbohydrate and 2000 calories. The results fail to reveal any uniform law of insulin requirement based on either the former tolerance, the body weight, the presumable basal or total metabolism, or any other single factor. In advance of the discussion in the following section, it may be well to state here that irregularities were encountered chiefly as luxus diets were approached. For example, patient No. 3 was later placed on 3000 calories and was made obese at a weight of 123 pounds. At this weight her insulin requirement is in the neighborhood of 60 units, even after a return to a 2500 calory diet. This requirement is expected to fall as her weight is lowered by reduced diet and increased exercise.



### 5. *Factors Governing the Insulin Requirement.*

When the idea of a fixed quantitative relation between insulin and glucose is disproved, and the observed variations between different individuals and different conditions are pointed out, practically all chemists and physiologists respond with the remark, "But there must be a quantitative relationship of some kind." Undoubtedly there is an accurate quantitative adjustment of insulin production to the needs of the body, but consideration of the factors which are already known to modify this need will show the difficulty of formulating any uniform law.

(a) *Severity of the diabetes:*—Comparisons between different diabetics demand some accurate method of estimating the relative severity of their cases, which in essence means the insulin production by each patient's pancreas. No fully satisfactory method is available, and cases which seem to be maximally severe according to their symptoms and D:N ratios under one plan of treatment may appear considerably milder under some more efficient treatment. The increased tolerance afforded by a certain dose of insulin may seem greater in a mild than in a severe case. This uncertainty concerning severity has nothing to do with the absolute insulin requirement of the body, but it is one of the difficulties in the way of the practical determination.

(b) *Infection:*—There is sufficient evidence that infection increases the insulin requirement. An explanation apparently cannot be found in a simple increase of metabolism, because the higher requirement is apparently encountered in cases with no marked elevation of metabolism, and furthermore it is not a general rule that increased metabolism necessarily increases the insulin requirement.

(c) *Acidosis:*—Regardless of any disputes concerning a specific raising of metabolism by acidosis, it is certain that the energy exchange is not multiplied in the degree to which the insulin requirement is often multiplied by acidosis. Speculation may suggest that in acidosis the body is full of half-burned metabolic products, which must be consumed by the aid of large supplies of insulin in order to clear up the intoxication. The only products of this kind known to be present in any large quantities are the acetone bodies. If it be true that insulin is used directly for the combustion of acetone bodies, a powerful argument is furnished in favor of its direct use in ordinary fat metabolism. The

question resolves itself into the same dilemma that exists for the action of insulin in general: is insulin used directly for burning the lower fatty acids? or do these acids somehow increase the amount of insulin that is needed for burning or storing glucose?

(d) *Food*.—Sufficient complexity is created by the fact that carbohydrate, protein, fat, alcohol, and presumably all energy carriers affect the insulin requirement. But still greater confusion is introduced by the evidence that different quantities of insulin may be required for the identical food mixture when burned under different conditions, as noted in the following paragraphs.

(e) *Metabolism*.—As the combustion of food substances increases the consumption of insulin, the reduction of total metabolism in fasting is probably one of the important factors in the control of diabetes by this means. As already stated, however, attempts to establish a rule correlating the insulin requirement with either the basal or the total daily metabolism encounter chiefly exceptions. Increase or reduction of the body fat seems to affect the insulin requirement disproportionately to the influence on the energy exchange. An athlete has a higher metabolism than a person of the same height and weight with small muscles and much adipose tissue, but the insulin requirement of the athlete would probably be lower with the identical diet. A diet which supplies the same amount, or actually less than the amount of food catabolized in fasting, increases the insulin requirement as compared with fasting in severe diabetes. Fasting or undernutrition raises the tolerance or reduces the insulin requirement of the diabetic, even when carried to a point which may sometimes not reduce or actually increases the total metabolism. High protein diets do not demonstrably increase the insulin requirement by their specific dynamic action.

(f) *Muscular exercise*.—Muscular labor not only increases the total energy consumption for the day but also, when sufficient in amount and duration, tends to raise the basal metabolism. At the same time it reduces the insulin requirement, just as it has long been known to raise the tolerance of patients, except those who were almost bankrupt in insulin owing to the severity of their diabetes.<sup>34</sup> The interpretation may be expressed in the statement that the muscles make more efficient use of a fixed supply of insulin, or metabolize a given supply of food with a smaller quantity

of insulin, when they are under the stimulus of active work or when their tonic contraction is strengthened as a result of previous work. The most plausible explanation, however, is that exercise is chiefly a form of undernutrition. With an unchanged food supply, the increased food consumption by exercise directly or by the higher basal metabolism with stronger muscular tone reduces the need for insulin, as compared with a condition of rest in which the food materials tend to accumulate.

(g) *Body weight*:—This is the most complex factor of all. Consideration of it starts from the postulate that the pancreas of a mouse could not possibly supply the body of an elephant; that in addition to the quantities of food to be metabolized, there must also be some relation between the quantity of insulin and the number of body cells to be supplied with insulin. Furthermore, the bodily composition is not uniform, but may be divided for the present purpose into the bones, the adipose tissue, and the muscles and viscera. If the bones, or at least their mineral portion, can be considered as inert, the same is not true of the adipose tissue, the formation and maintenance of which evidently calls for maximal quantities of insulin. This fact agrees with other evidence that insulin is not concerned in any internal protoplasmic activities of muscular or visceral cells (as represented, for example, in Folin's conception of endogenous metabolism), but rather with exogenous metabolism in the sense of the total food supply and nutritive level of the body. It may be mentioned in the same connection that reductions of the body mass of dogs by surgical amputations seemed to have a surprisingly small influence upon their diabetes as compared with the reduction of weight by fasting.<sup>35</sup>

Two features in connection with weight may be considered as having preeminent importance.

Age is one of these, because of the instructive comparisons which it affords between the widely different body sizes of children and adults. When all necessary allowances are made for the severity of the diabetes or the insulin supply from the patient's own pancreas, the observations already recorded show decisively that the minimum insulin requirement of children is lower than that of adults, but the requirement per kilogram of weight is considerably higher. The higher requirement per kilogram may be connected not merely with the higher metabolism in the catabolic sense, but also with the stronger anabolic or

growth tendency. Attempts to compare the requirements for various diets, either absolutely or per kilogram, are open to wide errors. In general, within certain limitations, children with their smaller body mass seem to require less insulin for the assimilation of the identical diet or even for a higher diet.<sup>36</sup> The best example is probably the 3-year-old child No. 918, who had such severe diabetes that her tolerance was not above 100 calories, but who became able to tolerate 1200 calories with 12 units of insulin per day, while the adult, No. 1286, with apparently no greater severity of diabetes, required over 15 units per day for a diet of 600 calories, and another adult (No. 1054) required at least 16 units for 1000 calories. It seems probable that the insulin requirement of very large adults may be found perceptibly larger than the requirement of very small adults under comparable conditions, but no definite calculations of this kind have been attempted because the conditions are hard to establish.

The second feature of outstanding importance is the general nutritive level. The greatest difficulty in attempting to estimate differences in insulin requirement according to differences of body mass consists in the fact that a diet which is only a maintenance or sub-maintenance ration for a large adult may be a *luxus* ration for a small adult or child, and here the tendency to increase weight and store up fat introduces a variable which is often greater than the one which is under study. The best illustration is furnished by the two children, No. 989 and No. 1034 (Table 23). They were closely comparable in age and height, and the boy, No. 1034, clearly had the more severe diabetes of the two. The boy was kept on approximately 800 calories, which permitted a gratifying gain in strength but only a slight gain in weight, and his condition was easily kept under control with 8 units of insulin per day. The girl, No. 989, was placed on high diets, which made her noticeably obese, and her insulin requirement accordingly rose to 70 units per day, which is the highest dosage given regularly to any patient in the entire series. Her final diet was 80 gm. protein, 100 or 150 gm. carbohydrate, and 2000 calories, and her weight was 61 pounds. Her insulin requirement was higher than that of adults who weighed two or three times as much, and who received considerably higher diets, merely because her diet was a *luxus* ration for her small size and caused her to accumulate adipose tissue. The boy, No. 989, was finally placed on the same diet as the girl, but his weight was only 40 pounds, and he there-



fore required only 24 units of insulin in order to assimilate this diet either without glycosuria or with no greater sugar excretion than that of the girl. Thus we have the anomaly that the child with the more severe diabetes required only about one-third as much insulin for the assimilation of the identical diet. There is nothing surprising in this fact to those who understand the influence of body weight upon diabetes, and there is no doubt that as the boy is allowed to gain weight his insulin requirement will rise, unless a gain in his own pancreatic function interferes. The tremendous burden placed upon the pancreatic island function by high calory diets is thus strikingly shown, and the influence of gluttony and obesity in provoking diabetes in persons predisposed by some degree of pancreatic injury is made more clearly comprehensible by being given a numerical expression.

It is by no means certain that the above known factors are the only ones which affect the insulin requirement, but they represent a sufficient number of variables to give pause to anyone who hastens to formulate arbitrary general laws. On the other hand, empirical rules are established for many phenomena which are fully as complex in character and control. Clinical experience already suggests that certain types of patients require approximately certain doses of insulin for certain diets. It seems possible that a more accurate basis may be obtained with the aid of respiration studies. The insulin requirement of depancreatized animals should be determined with fair ease, and some sort of a basic standard should be possible to establish for patients whose diabetes is so nearly "total" that their insulin production is practically negligible. Observations can then be made of the effect when the chosen standard condition is varied by single changes, such as the introduction of a certain number of grams of carbohydrate or of fat. The work of those who have sought to measure insulin dosage in terms of carbohydrate is not wasted, for the constancy of their results has been practically dependent upon the fairly constant conditions under which they have administered their carbohydrate. Their mistake has consisted in drawing broader conclusions than their narrow experimental conditions would support, and a new interpretation is necessitated by the proof that under similarly standardized conditions the influence of fat is as plainly demonstrable as that of carbohydrate. It seems probable that the insulin requirement of the organism will have to be measured chiefly in terms of total calories, with

certain allowances for the relative proportions of carbohydrate, protein and fat, and with careful avoidance of interfering influences.

### *Conclusions.*

1. The insulin requirement of the organism is governed not only by carbohydrate but also by fat and all other elements entering into the diet or metabolism.

2. It remains uncertain whether insulin is directly concerned in total metabolism, or whether it is specifically related to the assimilation of glucose alone and only in some secondary or indirect manner with the metabolism of other foods. It can only be said that the body cells somehow require insulin for their nutrition and consume it at a rather rapid rate in their life processes. Its anabolic is probably as important as its catabolic function.

3. The minimum insulin requirement compatible with sugar-freedom can be crudely estimated in depancreatized animals and in patients with maximally severe diabetes in a state of extreme inanition. This requirement may apparently be as low as 4 units per day in a young child, but is probably 12 units or more for an adult. Any diet sufficient for maintenance of life even on the lowest plane of nutrition probably requires a doubling of these figures. The need for insulin is related quantitatively to the body mass as well as to the amount of food to be metabolized. Under comparable conditions of nutrition, the insulin requirement of children is lower absolutely but higher per kilogram than that of adults.

4. The maximum insulin requirement is created by temporary emergencies such as infection or acidosis, from causes which are not understood. The maximum ordinary requirement is created by luxus diets and increases of weight. Special emphasis should be given to two facts: first, the increased need for insulin resulting from an increase of adipose tissue is fully as great as, and probably greater than, that which is created by an increase of active protoplasm in the muscles or viscera; second, this increased need not only occurs during the building up of this new tissue but also continues undiminished as long as the extra tissue is kept. It must therefore be concluded that insulin is used not only for the upbuilding but also for the maintenance of the body mass. The exaggerated insulin requirement with luxus diets and obesity explains the known diabetogenic influence of these conditions.



5. The experiments showing the influence of fat, total calories and body weight upon the insulin requirement confirm in all details the investigations upon which the undernutrition treatment of diabetes was founded. The theoretical position is thus strengthened on both sides. On the one hand, the undernutrition treatment is justified as against opposed dietary proposals. On the other hand, the exact correspondence of the results with insulin to the former modifications of tolerance by diet supports the belief that insulin is the true internal secretion of the pancreas and that diabetes is due solely to a deficiency of insulin.

## REFERENCES.

1. Allen, F. M., and Sherrill, J. W. *J. Metabol. Research*, 1, 1922, 377-434. Clinical observations on treatment and progress in diabetes.
2. *Monograph No. 11, Rockefeller Institute*, 1919. Total dietary regulation in the treatment of diabetes. Chapter I.
3. *Trans. Assn. Amer. Physicians*, 30, 1915, 338-340.
4. Woodyatt, R. T. *Arch. Int. Med.*, 28, 1921, 125-141. Objects and method of diet adjustment in diabetes.
5. Newburgh, L. H., and Marsh, P. L. *Arch. Int. Med.*, 26, 1920, 647-662; *ibid.* 27, 1921, 699-705. The use of a high fat diet in the treatment of diabetes mellitus.
6. Marsh, P. L., Newburgh, L. H., and Holly, L. E. *Arch. Int. Med.*, 29, 1922, 97-130. The nitrogen requirement for maintenance in diabetes mellitus.
7. Mosenthal, H. O., and Clausen, S. W. *Arch. Int. Med.*, 21, 1918, 269-281. The maintenance diet in diabetes mellitus as determined by the nitrogen equilibrium.  
Mosenthal, H. O., and Harrop, G. A. *Arch. Int. Med.*, 22, 1918, 750-758. The comparative food value of protein, fat and alcohol in diabetes mellitus as measured by the nitrogen equilibrium.
8. Fulton, F. T. *Rhode Island Med. J.*, 4, 1921, 143-145. Some radical changes in the treatment of diabetes mellitus.
9. Newburgh, L. H., and Marsh, P. L. (5). Also *Trans. Assn. Amer. Physicians*, 37, 1922, 117-165. Further observations on the use of a high fat diet in the treatment of diabetes mellitus.
- 9a. Allen, F. M. *Ibid.*, p. 162. Discussion.
10. Geyelin, H. R., and DuBois, E. F. *J. Amer. Med. Assn.*, 66, 1916, 1532. A case of diabetes of maximum severity with marked improvement; a study of blood, urine and respiratory metabolism.  
Jonas, L., and Pepper, O. H. P. *J. Amer. Med. Assn.*, 68, 1917, 1896. Acute diabetes with enormous elimination of nitrogen: report of case with at least temporary recovery.  
Fitz, R., and Bock, A. V. *Quart. J. Med.*, 12, 1919, 307. Study of a case of diabetes mellitus treated by the Allen method.

11. Joslin, E. P. *Trans. Assn. Amer. Physicians*, 37, 1922, 333-336. The urinary nitrogen excretion in diabetes.
12. Joslin, E. P. *Treatment of diabetes mellitus*, Lea and Febiger, 1917.
13. Allen, F. M., and DuBois, E. F. *Arch. Int. Med.*, 17, 1916, Part II, 1010-1059. Metabolism and treatment in diabetes.
14. (2), p. 383.
15. Allen, F. M. *Studies concerning glycosuria and diabetes. Harvard University Press*, 1919. Chapter I.
16. Atkinson, H. V. *J. Metabol. Research*, 1, 1922, 565-607. The transformation of protein into fat and fat into carbohydrate in the body.
17. Wilder, R. M. *J. Amer. Med. Assn.*, 78, 1922, 1878-1884. Optimal food mixtures for diabetic patients.
18. Campbell, W. R. *J. Metabolic Research*, 2, 1922, 606-635. Ketosis, acidosis and coma. Treatment by insulin.
19. Allen, F. M. *Amer. J. Med. Sci.*, 160, 1920, 781; *ibid.*, 161, 1921, 16, 165 and 350. *Amer. J. Physiol.*, 54, 1920-1921, 375, 382, 425, 439 and 451. *J. Metabol. Research*, 1, 1922, 619. Experimental studies in diabetes. Series II.
20. Allen, F. M. *Amer. J. Med. Sci.*, 153, 1917, 313. The role of fat in diabetes.
21. (2), Chapter VI.
22. Le Clercq, F. S. *J. Metabol. Research*, 1, 1922, 307. Overnutrition with fat and alcohol in severe diabetes.
23. Le Clercq, F. S. *J. Metabol. Research*, 2, 1922, 39. Further experiments with high fat diets in diabetes.
24. Allen, F. M., and Wishart, Mary B. *J. Metabol. Research*, 1, 1922, 281. Alcohol in the diabetic diet.
25. Fuller, L. S. *J. Metabol. Research*, 1, 1922, 609. The immediate influence of alcohol ingestion upon diabetic glycosuria and blood sugar.
26. Winter, L. B., and Smith, W. *Brit. Med. J.*, 1923 (1), 12-13. On a possible mode of causation of diabetes mellitus. Also *J. of Physiol.*, Dec. 1922, p. 100.
27. Weeks, Renner, Allen, Wishart. Publication forthcoming in this *Journal*, February, 1923.
28. von Noorden, C. *Der Diabetes mellitus*, Berlin, 1912.
29. Geelmuyden, H. Chr., *Ztschr. physiol. Chem.*, 73, 1911, 176-191. Ueber das Verhalten der Acetonkörper in intermediären Stoffwechsel.
30. Geelmuyden, H. Chr. *Die Neubildung von Kohlenhydrat im Tierkörper*. München, 1923.
31. Dakin, H. D., and Dudley, H. W. *J. Biol. Chem.*, 14, 1913, 423-431; 15, 1913, 463-474; 16, 1913-1914, 505-513. On glyoxalase. *Ibid.*, 18,

- 1914, 29-51. The formation of amino- and hydroxy-acids from glyoxals in the animal organism.
32. Joslin, E. P. *J. Amer. Med. Assn.*, 76, 1921, 79-84. The prevention of diabetes mellitus.
33. Bliss, S. W. *J. Metabol. Research*, 2, 1922, 385-400. Effects of insulin on diabetic dogs.
34. Allen, F. M. *Amer. J. Med. Sci.*, 161, 1921, 165. The internal pancreatic function in relation to body mass and metabolism. 3. The effects of exercise.
35. Allen, F. M. *Amer. J. Med. Sci.*, 161, 1921, 16. The internal pancreatic function in relation to body mass and metabolism. 2. Changes in assimilation by alterations of body mass.
36. Allen, F. M. *Amer. J. Physiol.*, 54, 1921, 439-450. The internal pancreatic function in relation to body mass and metabolism. 8. The influence of extremes of age upon the production of diabetes. (Puppies are not more susceptible to diabetes from partial pancreatectomy than adult dogs.)

## POSSIBLE SOURCES OF INSULIN\*

By C. H. BEST, M. A., AND D. A. SCOTT, M. A.

Since the presence of insulin was discovered by the work of Banting and Best<sup>1</sup> in the pancreas of animals and its preparation for use in man satisfactorily worked out with the assistance of Dr. Collip<sup>2</sup> various investigators have sought for its presence in other materials. In January, 1922, Doctors W. P. Warner, W. B. Dixon and C. S. Dixon<sup>3</sup> of the University of Toronto obtained, in several experiments, a substance from yeast capable of lowering the blood sugar and diminishing the sugar excretion of diabetic dogs. Later in the year Dr. J. B. Collip<sup>4</sup> demonstrated an insulin-like substance in the tissue of the clam. Professor J. J. R. Macleod,<sup>5</sup> in whose laboratory the discovery of insulin was made and under whose direction the physiological work on this material was fully developed, was successful in preparing insulin from the principal islets of certain boney fishes. In November, 1922, during the course of conversation with Dr. R. T. Woodyatt, in which the mechanism of the action of insulin was discussed, the idea presented itself that a hormone analagous to insulin might be present wherever glucose is metabolised, i. e., it might be present in plants. Arrangements were immediately made with Professor R. B. Thompson of the Department of Botany of this University to secure a supply of vegetable material.

Dahlia tubers and potatoes were first investigated on January 1st, 1923. The results were at first inconclusive. The work of Winter and Smith<sup>6</sup> in the Biochemical Laboratory at Cambridge by which they were able to conclusively demonstrate an insulin-like substance in yeast stimulated us to more intensive work on vegetable matter. This latter work has been more successful and we have been able to demonstrate a substance in potatoes, rice, wheat, beet roots and celery, which when administered to normal rabbits causes a marked lowering of blood sugar. We submit protocols of two typical experiments:

\* A preliminary communication from the Insulin Division, Connaught Antitoxin Laboratories, University of Toronto.

I. 2500 grams of germinating potatoes were finely minced. Alcohol made 0.35 per cent. acid with hydrochloric was added to the mince to make a concentration of approximately 70 per cent. alcohol. The mixture was filtered and the filtrate concentrated in vacuo. The residue was chilled and a slight precipitate settled out. This precipitate was taken up in acid water (Ph. 2.2) and the resulting solution injected subcutaneously into a rabbit weighing 1700 grams. The normal blood sugar was 0.098 per cent. The level was reduced to 0.066 per cent., 0.060 per cent. and 0.054 per cent. in 1, 2 and 4 hours respectively. The filtrate from which the precipitate was obtained was also effective in lowering the blood sugar of rabbits—one-tenth of the concentrated filtrate caused the blood sugar of a normal rabbit, weighing 1,000 grams to be reduced from 0.092 per cent. to 0.072 per cent. in two hours. The first extracts from potatoes were made in January, 1923.

II. 200 grams of rice were extracted for 18 hours with one litre of 80 per cent. alcohol, made 0.35 per cent. acid with hydrochloric. The solution was filtered and concentrated in vacuo. The residue was injected subcutaneously into a rabbit weighing 1400 grams. The normal blood sugar contained 0.130 per cent. dextrose. The blood sugar level at 1, 2, 4 and 6 hours was 0.066, 0.54, 0.054, 0.130 per cent. This experiment was repeated several times with similar results.

The experiments tend to show that a chemical body resembling insulin, so far as the effect upon the blood sugar of normal rabbits is concerned, is present in vegetable material. Obviously, many further observations are required before it can be definitely stated that this substance is insulin. We are engaged in this work at the present time.

In collaboration with Doctor F. G. Banting\* the authors have been able to obtain an insulin-like material in considerable quantities from the blood of the ox, dog, rabbit and man. Approximately thirty experiments in which samples of blood have been treated with the object of extracting insulin have yielded positive results. Full details of this work will be published shortly.

It is a pleasure to acknowledge our indebtedness to Mr. A. S. Wall for his assistance in this work.

---

\* Department of Pharmacology, University of Toronto.

## BIBLIOGRAPHY.

1. Banting, F. G., and Best, C. H. *Journal of Laboratory and Clinical Medicine*, 1922, Vol. XVI, 251.
2. Banting, Best, Collip and Macleod. *Proceedings of the Royal Society of Canada*, May, 1922.
3. Warner, W. P., Dixon, W. B., and Dixon, C. S. Unpublished.
4. Collip, J. B. *Proceedings, Journal of Biological Chemistry*, February, 1923.
5. Macleod, J. J. R. *Journal of Metabolic Research*, August, 1922.
6. Winter and Smith. *Journal of Physiology*, 57 P. 100, 1922.  
Nature, March 10, 1923.





# THE EFFECT OF GERMANIUM DIOXIDE UPON THE BLOOD.

By JOHN HUGHES MULLER, M.A., Ph.D., AND

MIRIAM STEWART ISZARD, M.A.

*From the John Harrison Laboratory of Chemistry and The School of Public Hygiene of The University of Pennsylvania, Philadelphia, Pa.*

The present investigation was undertaken in furtherance of a previous study concerning the erythropoietic action, cumulative effect, and elimination of germanium, publication of which appeared in the American Journal of the Medical Sciences.<sup>1</sup> This communication is, in the main, an attempt to account for the physiological effect which germanium preparations have upon the animal system, and is particularly concerned with the recognition and localization of germanium in the form constituents of the blood of animals treated with solutions of the dioxide of this element. In addition, certain facts have been accumulated which have bearing upon the saturation capacity or toleration of the blood for germanium dioxide and throw some light on the proper regulation of the dosage of germanium preparations required for maximum physiological effect. Methods for the preparation of germanium dioxide solutions suitable for clinical application, and some of the properties of these solutions are also given.

The experimental work which follows is divided into three parts:

1st. Chemical analysis of the form constituents of the blood of animals subjected to treatment with germanium preparations.

2nd. A comparative spectroscopic examination of the bloods of normal animals with the bloods of those treated with germanium dioxide.

3rd. Preparation of germanium dioxide solutions for clinical use.

## *Chemical Analysis of the Form Constituents of Blood of Treated Animals.*

The animals used in this phase of the work were male rabbits of approximately 2500 gm. body weight. In all cases the germanium dioxide was given by intraperitoneal injection of an aqueous solution of the

<sup>1</sup> Muller and Iszard, *Amer. Jour. Med. Sci.*, Vol. CLXIII, No. 3, Mar., 1922.

dioxide containing 0.00514 g. of the anhydrous oxide per cubic centimeter of solution. Before injection such solutions were made isotonic by the addition of 0.5 g. of sodium chloride for each 100 c.c. of the solution and also adjusted to a P.H. value of 7.5 by introduction of normal sodium hydroxide solution—drop by drop, until the weakly acid solution became faintly alkaline to litmus.

Chart No. 1 which follows, indicates the time of dosing and the amount of the dioxide administered in milligrams in the case of rabbits 1, 2, 3, and 4.

CHART NO. I.

Showing Dose of Germanium Dioxide in mgs. and times administered.

No. of Rabbit.	Days on which Ge O <sub>2</sub> was administered.	Amount of Ge O <sub>2</sub> in mgs.
1.	1st	128.5
	2nd	154.2
	4th	128.5
	5th	128.5
	6th	128.5
		668.2 total dose.
2.	1st	102.8
	2nd	51.4
	3rd	102.8
	5th	51.4
	6th	51.4
		359.8 total dose.
3.	1st	102.8
	2nd	51.4
	3rd	102.8
	5th	51.4
	6th	51.4
	8th	102.8
	9th	102.8
	12th	51.4
	13th	102.8
		719.6 total dose.
4.	1st	234.2
	2nd	234.2
		468.4 total dose.

These animals were anesthetised and bled to death from the jugular vein, the blood being collected in paraffin lined flasks containing 50 c.c. of physiological saline solution. The combined weight of flask and saline solution being known, the total blood sample was determined by re-weighing. During the operation of collecting samples, the containers

were kept chilled by packing them in ice. The blood drawn was prevented from becoming oxygenated by collecting in an atmosphere of carbon dioxide, and as soon as the weight of the total blood sample was determined the sample was divided into two equal volumes by introduction into calibrated, paraffin lined centrifuge tubes of known weight. Into one of these tubes carbon dioxide was introduced, and when filled nearly to capacity with the blood sample it was sealed off to prevent contact with air. The other tube containing an equal volume of the original blood sample was treated with oxygen gas to saturation and allowed to remain open to air. Both tubes, the open one containing oxygenated blood and the sealed tube containing the same quantity of reduced blood, were immediately centrifuged to separate the cells and plasma of each. The supernatant fluid consisting of blood plasma and physiological salt solution was in each case pipetted off as far as possible and the residue of cells from the reduced and oxygenated sample separately weighed. Washing of the residue of cells was not carried out for two reasons—first because the total amount of germanium in the whole blood was small and the accuracy of the result therefore not altered within the analytical limit of error by the small amount of diluted plasma remaining with the cells; second because contact with air was to be avoided. From the above procedure the weights of the form constituents of the two blood samples were obtained, giving four samples to be analyzed for each of the rabbits treated, namely:

1st, cells of the venous blood; 2nd, plasma of the venous blood; 3rd, cells of the oxygenated blood; 4th, plasma of the oxygenated blood.

These gross constituents of the blood were analyzed for their separate germanium content. The analytical procedure was practically the same as that outlined for the estimation of germanium in blood and other organic mixtures, in a previous publication.<sup>1</sup> This method was based upon the volatility of germanium tetrachloride in a stream of chlorine, and distillation of the tetrachloride from an aqueous solution containing hydrochloric acid. The destruction of the organic matter present was accomplished in the wet way by the action of free chlorine in the presence of hydrochloric acid, under circumstances which prevented any loss of the germanium. The germanium was finally obtained by precipitation of the sulphide, which was converted to oxide, in which condition it was weighed.

Results of these analyses are shown in Chart No. 2.

The results indicated (Chart 2) show that in the case of the venous blood there is a localization of the germanium in the cells rather than in the plasma, while in the case of the same blood oxygenated in vitro the reverse situation is observable. It is to be noted that in the case of the reduced blood the germanium content of the cells was in most cases about twice that of the plasma. In the oxygenated portions of these same blood samples much of the germanium previously associated with the



cellular constituents of the blood must have left the cells and passed into the plasma, for after absorption of oxygen the ratio of germanium in the cells to germanium in the plasma becomes roughly one to one, thus indicating a shift of position of the germanium brought about by the absorption of oxygen. This shift in the position of the germanium from the cellular constituents of the reduced blood to the plasma upon oxygenation may be accounted for perhaps in several ways—either by the existence of a complex consisting of hemoglobin combined with the lower oxide of germanium ( $\text{GeO}$ ), or by the incapability of a combination of germanium in the quadrivalent state with the oxyhemoglobin formed by absorption of oxygen. The former explanation will be later seen to be the more likely.

The authors realize that the errors in determining such small quantities of germanium are large, but nevertheless the concordance in the results clearly points to the above behavior. Moreover, the errors unavoidably introduced could not have seriously influenced the comparative values obtained for germanium inside and outside the cell bodies, though the absolute quantities of germanium given may all be a little low. Every analysis was carried out in exactly the same way.

As purely analytical data could not be further drawn upon to locate the position of germanium in the blood, it was convenient to formulate a working hypothesis to explain the change in position of the germanium in the venous and oxygenated bloods, which hypothesis rests not only upon the analytical results obtained but also upon certain marked analogies which are known to exist between the oxides of germanium and the far better known oxides of carbon which have the same chemical structure. In other words the periodic relationship which germanium bears to carbon is a valuable aid in this connection.

Carbon dioxide is of course eliminated from the venous blood when this reaches the lungs, with the resulting change to the scarlet arterial blood containing oxyhemoglobin. Carbon monoxide on the other hand forms a stable addition product with hemoglobin, stable enough to cause asphyxiation. Germanium forms two oxides of the same structure—( $\text{GeO}_2$  and  $\text{GeO}$ ), hence it is not unreasonable to suppose that these oxides might act in a somewhat similar manner. Germanium dioxide differs from its analogue carbon dioxide in that the former is a solid having no measurable vapor pressure, whereas the latter is a gas. It would



follow that the liberation of germanium from the cells of the reduced blood into the plasma of the oxygenated blood cannot result in pulmonary elimination of the germanium from the system as is the case with the dioxide of carbon. As a natural result the liberated germanium dioxide passing into the plasma could only form a solution of germanic acid and again return to the tissues of the body so dissolved. Elimination can take place through the kidneys, (as has been shown by previous work).<sup>1</sup> Germanic oxide is easily reduced to either metal or lower oxide and it is conceivable that it would be reduced in the tissues to germanous oxide, again combine with the hemoglobin and return to the lungs in the venous blood stream, completing the cycle.

Still other considerations favor the above suppositions, namely: the curious facts that germanic and carbonic acids have nearly the same hydrogen ion concentrations in their saturated aqueous solutions (the authors have determined this to be the case by the indicator method), and that these two oxides are fairly comparable in their respective solubilities in water.

The analytical results obtained are of course incapable of defining the state of oxidation of the germanium in the cells of the venous blood and it is not possible to determine this by any direct chemical measurement owing to the small amount of germanium present and the nature of the cell constituents. However, it is suspected that the monoxide of germanium, being much more basic than the dioxide, would be capable of combination with the feebly acid hemoglobin in the venous blood and this might lead to the formation of an addition product similar to the well known hemoglobin-carbon monoxide complex. Oxidation of this would then release the combined germanium as  $\text{GeO}_2$ , and the latter being an acid anhydride would dissolve in the plasma as the ortho acid.

Briefly, the supposition as to the behavior of germanium in the blood, which is in harmony with the analytical results, is as follows: The injection of germanium dioxide into the system first results in the absorption of germanium by the blood stream to the extent of 0.0065 to 0.014 per cent., depending upon the amount administered and the factor of elimination.<sup>1</sup> This is followed by reduction in the tissues of the body, with the resulting combination of the germanium in the lower state of oxidation with the hemoglobin of the venous blood. The venous blood when reoxygenated in the lungs causes the oxidation of the

bivalent germanium to the quadrivalent state ( $\text{GeO}_2$ ), and this oxide being the anhydride of an acid comparable to carbonic acid, but nonvolatile, remains in the plasma as the ortho acid, completing its cycle.

The above working hypothesis cannot be either confirmed or disproved by purely chemical methods and serves merely as a means of working out a different mode of attack which might perhaps result in the substantiation or destruction of some or all of the above assumptions. This leads to the most promising means of attacking the problem, namely, comparative spectroscopic analysis of normal and treated bloods.

*Spectroscopic Examination of the Blood of Normal and Treated Animals.*

*Apparatus:* The instrument used was a Hilger direct reading spectrometer. The instrument was calibrated by known bright line spectra through the range of the field of blood absorption spectra. As a convenient means of defining the position of the absorption bands of the blood samples, a solution of didymium nitrate of known concentration was used. This was sealed in a glass absorption cell (1 cm. depth) and the position of the edges of its absorption bands determined. This spectrum was photographed in conjunction with all blood spectra, at both the top and bottom of each plate.

The source of light was a 200 watt tungsten lamp fixed in position so as to give a constant light intensity as far as possible throughout the entire series of spectroscopic determinations. The slit of the instrument and all other adjustments were maintained as far as possible without change. The light intensity was arranged so as to require a time exposure of three minutes in order to reduce the error in this connection to a minimum. This was done by making a series of preliminary experiments with a 1 to 50 normal blood solution, and regulating the position of the source of light and adjusting the slit so as to give a negative of proper intensity in an exposure of this time interval. Short time exposures would not have given comparable spectra, hence the importance of this precaution.

The plates used were fresh W. & W. Panchromatic ( $3\frac{1}{4} \times 4\frac{1}{4}$  in.). Prints were made on contrast glossy Velox. Conditions for development, fixing, and printing were carefully regulated, all reagents were freshly prepared and constant temperatures maintained throughout.

All blood spectra were photographed through one centimeter depth of the solution, using blood of 1 to 50 dilution.

*Dosing of Animals:* The germanium dioxide solution was of the same concentration and the method of administration the same as employed in the work under chemical analysis of treated bloods.

Chart 3 shows time and amount of dosing, also time of collection of the blood samples.

The control rabbits and those to be treated were selected in pairs of very nearly the same body weight, and of the same sex.

Preliminary blood spectra were photographed to assure similarity of the control and the animal to be treated in the several experiments described, comparing the spectra of both oxygenated and reduced bloods of these animals before submitting one of the pair to treatment with germanium dioxide solution.

### CHART NO. 3.

Showing Doses of Germanium Dioxide given to Animals used in Spectroscopic Analysis.

Series No.	Time of dose.	Amount Ge O <sub>2</sub> given. mgs.	Time of Collecting Blood Sample.
0.	1st day.	102.8	1st day. (See Plate A & B Series O.)
	2nd "	102.8	2nd "
	3rd "	102.8	3rd "
	.....	.....	5th " (See Plate C, Series O.)
	.....	.....	6th " (See Plate D, Series O.)
	.....	.....	7th "
	.....	.....	8th "
1.	1st day.	257.0	1st day.
	3rd "	.....	3rd " (See Plate A & B, Series 1.)
	.....	.....	4th " (See Plates C & D, Series 1.)
2.	1st day.	102.8	1st day.
	2nd "	51.4	2nd " (See Plates A & B, Series 2.)
	3rd "	51.4	3rd "
	4th "	51.4	4th "
	5th "	257.0	5th "
	.....	.....	.....
	.....	.....	7th " (See Plates C & D, Series 2.)
3.	1st day.	154.2	1st day.
	3rd "	102.8	2nd "
	4th "	257.0	4th " (See Plates B, C & D, Series 3.)

*Method of Procedure:* On the days indicated in Chart 3, under collection of sample, the treated and normal animals were bled from the ear vein. The bloods were collected in flasks containing 100 c.c. of distilled water, flasks and contained water having been previously weighed. Approximately the same volumes of blood were drawn from each animal (2.5 to 3.0 c.c.) and the exact amounts taken then determined by reweighing the flasks. The samples were then diluted with the requisite amount of water to bring each to exactly 1 to 50 dilution. These solutions of 1 to 50, hemolyzed blood were shaken with excess of air and from aliquot portions of each the comparison of their absorption spectra was made. The spectra to be compared were photographed upon the same plate, the exposures of the treated and control being made one immediately following the other. It should be noted that conditions of developing and printing were necessarily the same in each case as both spectra were on the

same plate. All conditions of developing, fixing and printing and the use of reagents were standardized, however, so as to make not only comparable spectra on any single plate, but also to make all plates shown fairly comparable to each other.

After having obtained the absorption spectra of the oxygenated bloods, equal portions of these same samples were subjected to various reducing conditions and the resulting reduced bloods were spectroscopically compared.

In general three methods for reducing the oxygenated bloods were used: 1st. Equal volumes of the 1 to 50 diluted, oxygenated bloods were treated with carbon dioxide and allowed to remain in the atmosphere of this gas, care being taken to submit the control and treated bloods to the same conditions of pressure, temperature, and time of exposure. This means of reduction was applied in the bloods of series 0, 1, and 2. In series 3 the reduction of the oxygenated bloods was brought about by the action of bacteria (*Staphylococcus aureus*, advantage being taken of slower, selective absorption of oxygen in the absence of air.

Selection of the proper organism to effect the reduction was determined upon by previous experimentation on the germicidal action of germanium dioxide upon various bacteria. This preliminary work showed that *Staphylococcus aureus* was not affected by half-hour exposure in a saturated solution of germanic acid and hence this organism lends itself admirably to the reduction of oxyhemoglobin in the absence of atmospheric oxygen. In making tests of the bacterial reduction of the oxygenated bloods of series 3, a freshly isolated twenty-four hour culture of *Staphylococcus aureus* was used. The bloods of the control and treated animal were collected and diluted as previously outlined and a portion of each was used for determining the selective absorption of the oxygenated bloods; the remaining equal volumes of these blood samples were then filtered through a sterilized Berkefeldt filter and the filtrate collected in sterile filter flasks containing 30 c.c. of paraffin oil. 100 c.c. each of the normal and treated bloods were thus collected beneath thick layers of liquid paraffin. Both samples were then inoculated with 0.5 c.c. of a broth suspension of *Staphylococcus aureus* and the two were allowed to incubate at 37° C.

Observations were made from time to time to ascertain whether there was any difference in the degree of reduction of the normal blood as compared to the blood containing germanium throughout equal time intervals. As will be shown later the treated blood differed considerably in its behavior from the normal blood of the control animal.

As before mentioned, the reduced bloods were protected from air by a supernatant layer of paraffin oil, transference of the reduced sample to the absorption chamber was made with a sterile pipette under circumstances which prevented contact with air, and the sample removed was introduced into the absorption cell beneath a thick layer of paraffin oil.

3rd. Still another means of effecting reduction of the oxygenated bloods of control and treated animals was resorted to in a number of preliminary experiments, namely, the subjection of these to the action of



reduced pressure. The separate flasks containing the bloods of the control and treated animals were connected with each other by a "T" tube and the air nearly exhausted. They were allowed to remain *in vacuo* for some hours and when change occurred by gross inspection, the samples were examined for their respective absorption spectra.

It was noticed that blood of the normal or control animal always showed the change to the reduced hemoglobin spectrum before that of the animal treated with germanic acid. Many preliminary experiments were made in this way upon the bloods of both guinea pigs and rabbits, but as conditions of dosing and general technique in observing the spectra were at this time not so carefully regulated as in the series 0, 1, 2 and 3, only a single plate for the results obtained for the guinea pig and one plate for those found in the case of the rabbit are shown. (Series 4, Plates A<sub>1</sub> and B<sub>1</sub>.)

*Results Obtained by Spectroscopic Comparison of Normal Blood and That of Animals Treated With Germanium Dioxide.*

Five series of plates showing the spectra of normal bloods of the control animals, together with those exhibited by the bloods of treated animals, follow. In the plates of series 0, 1, 2, and 3 the spectrum of the normal animal appears first and is marked "N" while that of the germanium treated animal follows and is marked "GeO<sub>2</sub>." The compared blood spectra are photographed between two standardized didymium spectra placed at top and bottom of each plate. The wave lengths of these defined bands appear at the bottom of each series of plates.

Series 0. Plate A shows the initial similarity of the bloods of the two rabbits used in this series, the normal oxyhemoglobin spectra of the control and animal to be treated being almost identical. The two bloods were now converted to reduced hemoglobin through the action of carbon dioxide as explained above and the similarity of the reduced bloods also demonstrated, as shown in Plate B.

One of these animals was treated with germanium dioxide (for doses and times of administration consult Chart 3, Series 0). Following the initial dose of germanium, daily photographs were taken of the blood spectra of the control and treated animals, both of the oxygenated and reduced samples (all plates are of course not shown). It was not until the fourth day after the initial dose that any dissimilarity in the bloods of the treated and control animals was observed. This dissimilarity is shown in reduced bloods in Plate C, but no difference could be seen in the oxygenated bloods which had been compared immediately before C was photographed. On the fifth day after the initial dose the same condition obtained, as can be seen in Plate D. On the fifth day the oxyhemoglobin spectra had also remained the same as on the previous day and are therefore not reproduced here.

Series 1. The rabbits used in the foregoing series (No. 0) were put aside for a period of thirteen days and their bloods again compared before

SERIES 0

Plate A—Oxygenated blood

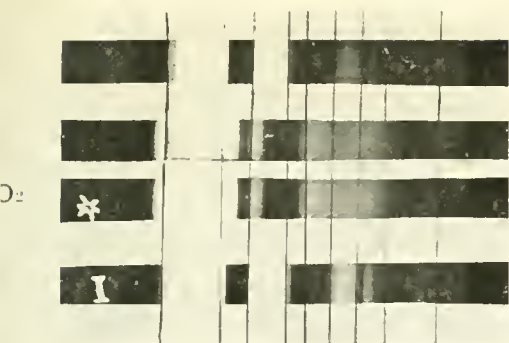


Plate B—Reduced blood

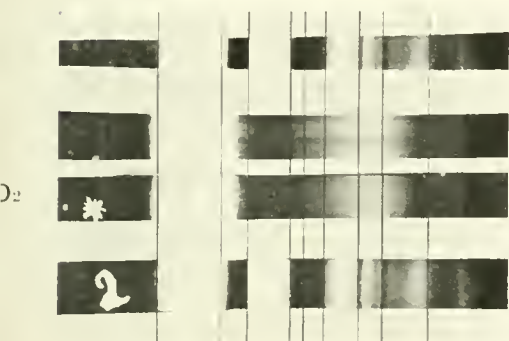


Plate C—Reduced blood

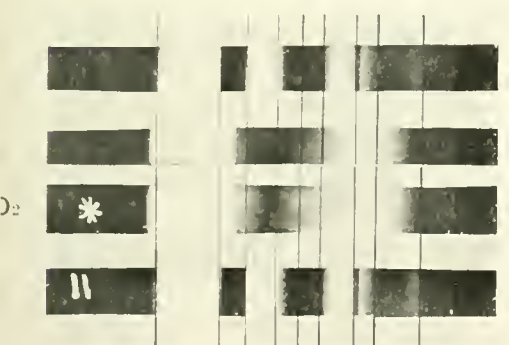
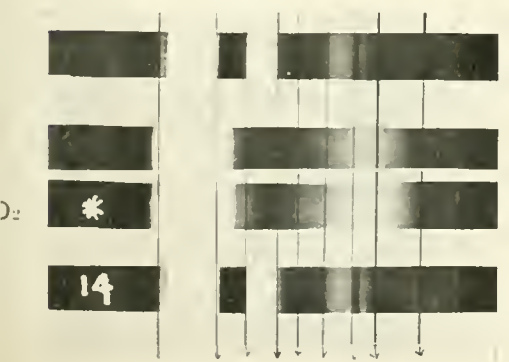


Plate D—Reduced blood



734.5  
388.4  
368.8  
325.5  
317.7  
307.4  
283.8  
270.5  
249.5

SERIES 1

125

Plate A—Oxygenated blood

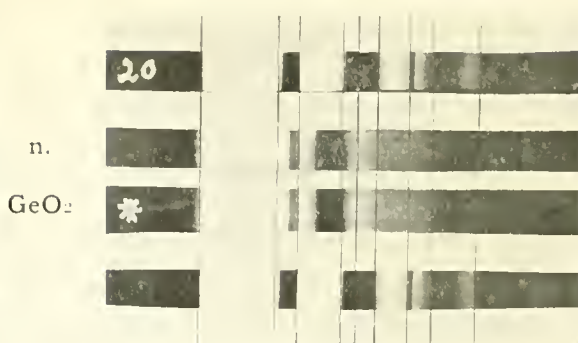


Plate B—Reduced blood

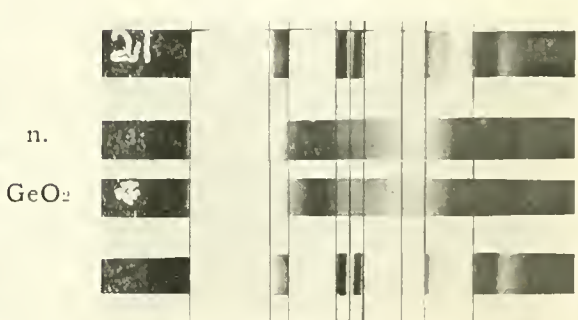


Plate C—Oxygenated blood

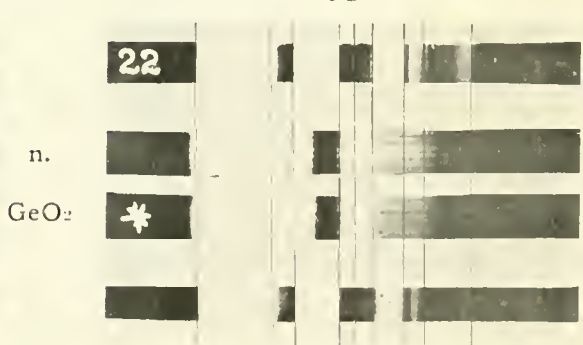
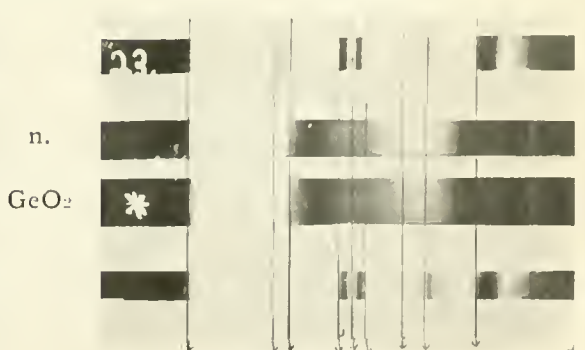


Plate D—Reduced blood



34.5  
388.4  
368.8  
325.5  
317.7  
307.4  
283.8  
270.5  
249.5





Plate A—Oxygenated blood

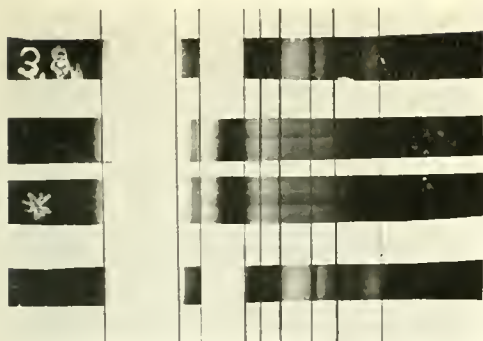


Plate B—Reduced blood

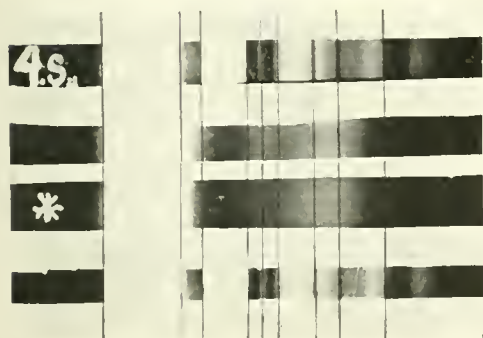


Plate C—Oxygenated blood

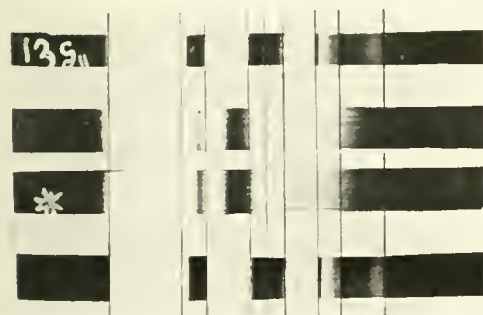


Plate D—Reduced blood

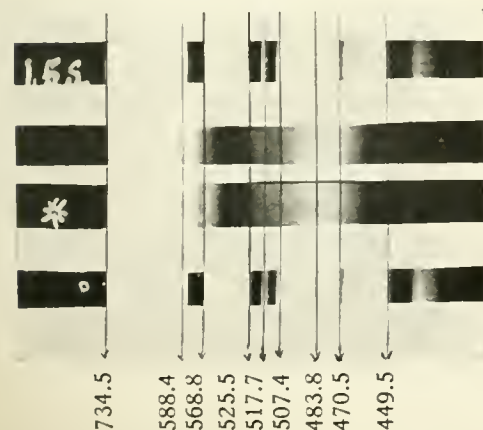


Plate A—Oxygenated blood

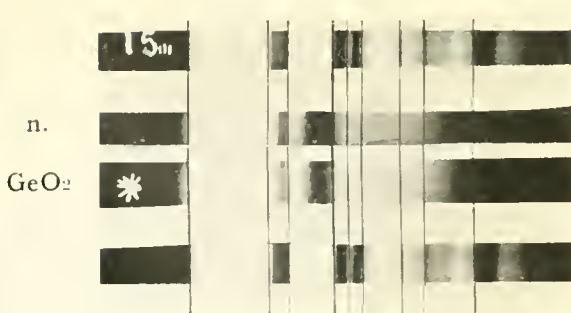


Plate B—Oxygenated blood

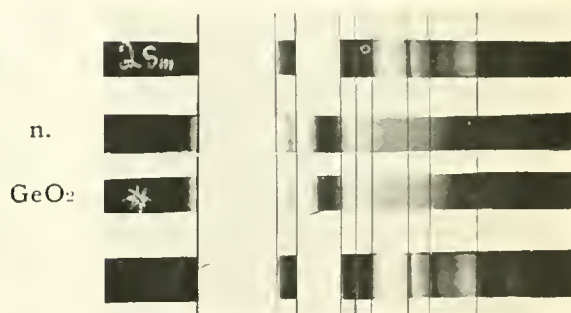


Plate C—Reduced blood

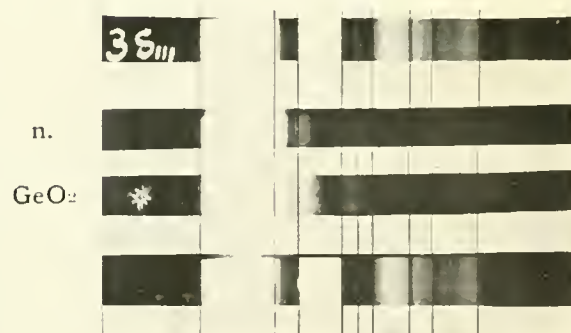
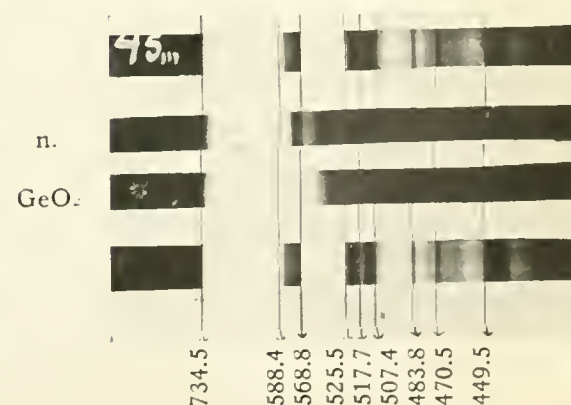


Plate D—Reduced blood





# SERIES 4

## Plate B<sub>1</sub>

Guinea Pig blood



## Plate A<sub>1</sub>

Rabbit blood



Illustrating effect of reduced pressure upon normal and treated bloods of guinea pig and rabbit.



further treatment of one of them with germanic acid. As far as the spectroscopic examination could show, these had regained their original similarity both in the reduced and oxygenated samples. One of these was treated with a single large dose of germanic acid, selecting the rabbit which was formerly used as the treated animal. (Consult Chart 3 Series 1.) Daily examinations of these bloods were again made, from which were observed two interesting facts: 1st. The same difference between the bloods of the control and treated animals appeared, and only in the reduced bloods as before. 2nd. The maximum difference was reached this time on the second day after the single dose, while the effect on the same animal when first subjected to the action of germanium showed change only after the fourth and fifth days after the injection. This seems to indicate that some germanium had remained in the blood stream, the animal only requiring a single retreatment to make an observable change in the selective absorption produced by the venous blood. See Plates A and B, Series 1.) On the third day after the dose the condition persists, as seen in Plates C and D of the same series.

Series 2. In these experiments a new pair of rabbits was used, selected as before in regard to sex, body weight and general condition. Their bloods were proved to have the same selective absorption both in the venous and oxygenated condition. (These plates were exactly similar and are not here reproduced.) One of these rabbits was treated with five successive doses of germanic acid (see Chart 3, Series 2) and the spectra of the venous and arterial blood compared daily with the control, as in previous series.

Plates A and B, Series 2, show that spectra taken on the first day after dosing do not vary appreciably from the normal either in the oxygenated or in the reduced bloods. Plates C and D, Series 2, however, plainly show that on the sixth day after the initial dose (second day after cessation of all dosing) the reduced samples differ strikingly from each other and again this difference appears only in the venous and not in the oxygenated blood. (Compare Plates C and D, Series 2.)

It should be noted that in all the above series of experiments the oxygenated bloods were reduced through the agency of carbon dioxide, the use of which has been explained under methods of procedure.

Series 3. Another pair of rabbits was selected, observing the same precautions as in previous series. One of these was treated with germanium dioxide in three doses as indicated in Chart 3, Series 3. The total amount of germanium dioxide amounted to 514.0 mg.

Plate A, Series 3, shows the spectra of the oxygenated blood of this animal and of the control previous to the initial dose. Plate B of the same series shows the spectra of the oxygenated bloods to be similar on the fourth day following the series of doses (see Chart 3, Series 3). It will be seen that these spectra are similar to each other, showing lack of change in the oxygenated bloods of treated and control animals.

The bloods of these animals were then reduced, but it should be noted that the reduction was carried out in a different manner than in the pre-



ceeding series 0, 1, and 2. That is to say, equal portions of the normal and treated oxygenated bloods were reduced by bacterial action, using a freshly isolated twenty-four hour culture of *Staphylococcus aureus*, which culture in broth suspension was used to inoculate the blood samples. Conditions were such as to prevent bacterial contamination, as explained under method of procedure.

Plate C shows the results of bacterial reduction of the oxygenated blood of the normal and control animals. This was taken on the fourth day after the initial dose of germanium dioxide and shows the effect twenty-two hours after the inoculation of the oxygenated bloods with the broth suspension of *Staphylococcus aureus*. Plate D shows the condition to be the same as in Plate C, but was taken seven hours later when the selective absorption of oxygen by the growing organism had been allowed to proceed still further.

It is observed that the maximum effect appears on the fourth and fifth day after the discontinuance of the dosing, which effect as in all other experiments takes place only in the reduced or venous blood; but moreover it can be seen that the difference between the spectra of the reduced bloods of the treated and control animal is more prominent in these bloods than in the previous series of experiments although the same qualitative distinction exists. This is explained by the fact that the reduction of the oxygenated bloods by carbon dioxide is so rapid as to be beyond the control of the experimenter, while the slower action of a growing culture of *Staphylococcus aureus* allows spectroscopic examination to be made at any stage of the reducing process. This is the reason why the bacterial reduction was used.

Series 4. The plates in Series 4 are representative of results obtained in a long series of preliminary experiments upon the bloods of both guinea pigs and rabbits, in which nine pairs of guinea pigs and three pairs of rabbits were used. In general the blood pictures showed the same general effect as seen in the four series described above. In the preliminary work, however, the oxygenated bloods were reduced by submitting them to reduced pressure. In all cases the blood of the animals treated with toxic doses of germanium dioxide resisted reduction to a greater extent than the blood of the control animals, resulting in the production of spectra much more closely resembling the selective absorption of oxyhemoglobin than that of reduced hemoglobin, easily formed in the case of the control.

Plate A<sub>1</sub> shows the absorption spectrum of the oxygenated blood of a guinea pig which had been treated with toxic doses of germanium followed by the spectrum obtained after exposing this blood to reduced pressure for some hours. The third spectrum on the same plate shows the effect of reoxygenating by shaking in air. In the fourth spectrum can be seen the normal reduction of the blood of the control animal which has been subjected to exactly the same treatment in regard to time of exposure to vacuum, temperature, etc., as the blood of the germanium treated animal.

Plate B<sub>1</sub> (Series 4) shows the difference in the behavior between the

oxygenated blood of a rabbit treated with a toxic dose of germanium and that of the normal blood of the control animal, both of which had been exposed to the same conditions of reduced pressure.

The spectra in Series 4 are simply offered to show that exposure of the oxygenated bloods to partial vacuum alone will result in production of different spectra in the normal and treated bloods, provided of course that bacterial contamination be avoided.

It is interesting to note that Stokes' reagent as well as other commonly used reducing agents, such as dilute alkali sulphite, entirely failed to show any difference between the bloods of normal and treated animals. The addition of these reagents resulted in the reduction of both normal and germanium containing blood, and upon spectroscopic examination no differences could be seen in their selective absorption. The failure of Stokes' reagent in this connection is important, as it indicates that the hemoglobin germanium complex probably present in the venous blood of the treated animals cannot be as stable as the additive compound formed by carbon monoxide with hemoglobin, as the latter resists the action of Stokes' reagent and is so recognized.

#### *Conclusions Drawn From Spectroscopic Analysis.*

Certain outstanding facts appear in all of the results of the outlined spectroscopic examination of blood containing germanium dioxide, as follows: 1st. The influence of germanium in the blood is perceptible only in the reduced or venous blood. 2nd. The difference from the blood of normal animals consists in a decidedly increased resistance to those influences which normally cause rapid reduction of oxyhemoglobin to reduced hemoglobin, namely, the action of carbon dioxide, the action of reduced pressure and the reducing action of oxygen-consuming bacteria. 3rd. The reduced blood of animals treated with germanium dioxide shows a characteristic spectrum which is totally different from that obtained in normal reduced blood, resembling the absorption spectrum but not identical with the latter.

It is believed that this selective absorption is due to the formation of a complex which germanium forms with the hemoglobin in the cells of the venous blood. This tallies with the results obtained in the analytical way, in which it appeared that the reduced or venous blood contained much more of the germanium within the cells than in the plasma (see Chart 2). Analysis showed also that after oxygenation, the blood then contained more germanium in the plasma than in the cells, or at least that much of the germanium previously in the cells passed into the plasma upon oxygenation of the reduced blood. This in a meas-

ure agrees with the fact that oxygenated bloods did not show any characteristic absorption phenomena to indicate the presence of germanium. It has been ascertained that a solution of germanic acid itself does not give any absorption spectrum and likewise demonstrated that oxygenated bloods containing as much as 0.014 per cent.  $\text{GeO}_2$  show no absorption bands other than those of normal blood; reduction, however, results in the production of a new absorption spectrum which must be due to the germanium present.

Is it not significant, since carbon monoxide forms a complex with hemoglobin which resists reduction and is spectroscopically recognizable, that the venous blood of germanium treated animals shows likewise a resistance to reduction and is also spectroscopically altered?

This is not proof that the germanium, in the venous blood, within the cells, is actually in the lower valence ( $\text{GeO}$ ), but it certainly makes this assumption very plausible. As before mentioned, the use of Stokes' reagent will not show any difference between normal blood and that containing germanium, and from this fact it is probable that the complex perhaps formed by germanium and the hemoglobin of the venous blood is less stable than the analogous carbon monoxide hemoglobin.

The characteristic behavior of germanium-containing blood is best seen in Plates C and D of Series 3. Exact measurements of the positions of these bands have not yet been made, though it is plain that the spectrum is not that of either reduced hemoglobin or of some oxyhemoglobin which had escaped reduction, the principal difference from the selective absorption of oxyhemoglobin being, the fading out of the well defined band in the region of 580 to 590 and the lack of definition in the other band on the side toward the violet near 550. The positions of the absorption bands were not studied more closely in the comparative study of normal and treated bloods here presented, but will be separately investigated later.

Finally, if germanium forms a complex in the cells of the venous blood, as seems to be indicated by both chemical analysis of the form constituents of the blood and spectroscopic examination, it is probably present as the lower oxide, in which an analogous behavior to the monoxide of carbon is exhibited. Upon oxygenation in the lungs the germanium monoxide takes up oxygen with the formation of the tetravalent germanium and

so passes into the plasma of the arterial blood. Here the germanium is no longer recognizable by spectroscopic examination, because it is then free as germanic acid in the plasma and hence cannot modify the normal spectrum of normal oxyhemoglobin. Germanic acid being nonvolatile remains in the plasma, returns to the tissues so dissolved and is then probably reduced to the lower oxide, as the reduced blood spectroscopically shows the presence of germanium associated with the hemoglobin in such a manner as to modify its normal absorption spectrum.

This of course suggests that the function of germanium in the blood is that of an oxygen carrier, which if true may explain the erythropoietic action of germanium and account for the condition of hyperemia produced in the bone marrow.

It has been the experience of the authors that a number of animals treated with toxic doses of germanium dioxide suffered marked loss in weight. Postmortem examination showed that the amount of fat normally present had almost completely disappeared. This consumption of fat may likewise be explained on the assumption of an increased oxygen exchange. If this be true there should be a measureable increase in the production of carbon dioxide by these animals. This suggests another line of investigation which ought to be carried out.

#### *Capacity of the Blood for Germanium Dioxide.*

Analytical results obtained in the recognition of germanium in the form constituents of the blood (Chart 2), and also a number of analyses of the whole blood of both guinea pigs and rabbits used in preliminary experiments, will bear out the statement that the blood seems to have a certain saturation capacity for germanium.

In the case of the rabbits used in the quoted analyses of form constituents of the blood, the doses were 668.2, 359.8, 719.6, and 468.4 mg., administered as seen in Chart 1, rabbits, 1, 2, 3, and 4, respectively.

In these animals, which were of nearly the same body weight (2500 gm.) the germanium present in the total blood of each animal may be calculated. The calculation, based on the assumption that one-thirteenth of the body weight is blood, shows the following number of milligrams of  $\text{GeO}_2$  to be present in the bloods of these animals in the same order: 33.6, 28.0, 18.5, 21.0. It is evident that the size of the dose and probably the method of



administration have little connection with the amount present in the blood stream. Converting these values to percentages of  $\text{GeO}_2$  in these bloods the following values are found: 0.010 per cent., 0.009 per cent., 0.006 per cent. and 0.007 per cent. In all bloods analyzed no greater quantity of germanium dioxide has been found than 0.014 per cent. and the smallest amount recovered was never less than 0.006 per cent., hence it is probable that the attempt to introduce larger amounts of germanium will meet with failure.

On the assumption that a man of average weight will respond to treatment with germanium dioxide in the same way as the rabbit and guinea pig, it can be calculated that to produce the maximum effect of this material upon man would probably require about 0.8 to 1.0 grams of the dioxide.

Considering the factor of elimination through the kidneys, it has been shown by previous work<sup>1</sup> that the administration of a single large dose results in a rapid elimination of much of the excess quantity in twenty-four to forty-eight hours.

It would then appear reasonable to suggest a certain mode of administration of germanium preparations for clinical study: 1st. Establish a toleration of the material by injection of small amounts daily, so as to reduce the necessity for excessive elimination, gradually increasing the dose daily as is commonly done with arsenical preparations. 2nd. Continue the treatment until a quantity of germanium has been given somewhat comparable to the required amount based on the body weight. This would be, as before mentioned, about 0.8 to 1.0 gm.  $\text{GeO}_2$ . The germanium dioxide solutions contain about 0.005 gm.  $\text{GeO}_2$  per c.c., and this would mean the distribution of 160 to 200 c.c. of the solution in a series of doses.

It will be seen that all the animals experimented upon received far larger quantities in proportion to their body weights than this, and did not show any definite signs of poisonous action, the only noticeable change being a slight loss in weight. For example, rabbit No. 3 (Chart 1) received in all 719.0 mg. Rabbit No. 2 (Chart 1) received only 359.8 mg. These doses, calculated in the same ratios for a man of 60 kg., would amount to 17.2 gm. and 8.62 gm. respectively. The margin of safety as far as toxicity is concerned thus appears obvious.

Germanic acid solutions of such large volume would naturally be more conveniently administered by mouth. This mode of

taking the material into the system is probably less rapid in its effect than hypodermic injection, but it has been shown that germanic acid taken by mouth is completely absorbed. This is apparently the case because doses taken by mouth result in elimination through the kidneys and not by way of the intestinal tract, both in the rabbit and in man.

*Suggested Method for Preparation of Germanium Dioxide Solutions for Clinical Investigations.*

The following method can be used for preparing solutions of the dioxide, which will remain stable indefinitely.

Place 5 gm. of the ignited dioxide, or 6.5311 gm. of the sulphide, in a liter flask with slightly less than a liter of distilled water. If the oxide is used as the starting product, digest at the boiling point until the oxide hydrates and dissolves to clear solution. If the sulphide is the starting product boil actively until all the hydrogen sulphide is expelled, replacing the water of evaporation from time to time. Boiling should be continued until no hydrogen sulphide can be detected in the escaping steam. This test may be carried out as follows: Moisten a piece of filter paper with a dilute sodium nitroprusside solution slightly alkaline with sodium hydroxide and introduce the moistened paper into the neck of the flask. A pink or violet color indicates the presence of even the smallest amount of hydrogen sulphide.

The solution whether prepared from the weighed oxide or the equivalent weight of sulphide is filtered if necessary, and after cooling to room temperature, diluted to exactly one liter. The solution will then contain 0.005 gm.  $\text{GeO}_2$  per c.c. and is ready for immediate use if taken by mouth.

For hypodermic application the weakly acid solution prepared as above should be adjusted to a pH value of 7.5, which condition is obtained by adding normal sodium hydroxide solution, drop by drop, until faintly alkaline to litmus.

The addition of 0.5 gm. of sodium chloride for each 100 c.c. of the above solution will yield a solution approximately isotonic with blood plasma.

Sterilization of the solution can be carried out in the autoclave or the solution can be boiled without altering the material in any way, but continued evaporation should be avoided or the water lost by boiling should be replaced before the solution is allowed to cool. Solutions more concentrated than 0.005 gm.  $\text{GeO}_2$  per c.c. are not stable, tending to deposit more or less of the difficultly soluble oxide on standing, which will not redissolve at any reasonable dilution.

Pure germanic oxide is usually prepared by the action of water upon the redistilled tetrachloride, in which the oxide separates as a snow white impalpable powder. It may be washed with cold water without much loss, to remove the hydrochloric acid split off by hydrolysis of the



tetrachloride. Prepared from the sulphide, by oxidation with nitric acid, the dioxide is denser but completely soluble in water. Difficulty is experienced in eliminating the last traces of sulphur even upon continued retreatment with nitric acid and subsequent ignition.

Germanium dioxide is fusible with difficulty, as the melting point is close to 1070° C. When fused, it is a glassy mass, brittle and translucent, but still remains soluble in water with no greater difficulty than the unignited oxide. Solution of either the fused or finely divided oxide is very slowly brought about in the cold, but digestion with boiling water will result in a complete solution of 5 gm. of the oxide in 800 to 900 c.c. of water in about an hour.

### *Summary and Conclusions.*

1. Analysis of the form constituents of reduced and oxygenated bloods of animals treated with germanium dioxide shows that in the venous blood there is a localization of the germanium in the cellular constituents rather than in the plasma. In the oxygenated or arterial blood, on the other hand, the germanium was found in greater quantity in the plasma than in the cells. As the same samples were used in both cases it was concluded that a shift of the germanium must have taken place upon passing from the venous to the arterial condition, which change could only have been caused by the presence of oxygen.

2. Analyses of the form constituents of the blood as well as the determination of the germanium content of the whole blood of a number of animals indicate that the blood can only absorb 0.014 per cent. of its weight of germanium dioxide, and that in no case was less than 0.006 per cent. of the oxide found in the blood of animals treated with this compound. It was concluded that 0.014 per cent. represents approximately the saturation capacity of the blood for germanium dioxide, which quantity seems to be independent of the amount administered; and assuming that the same holds true in the case of man, it can be calculated that 0.8 to 1.0 gm. would be sufficient to obtain the maximum effect in a person of average weight.

3. An hypothesis was formulated, based upon the results obtained by analysis and analogies existing between the oxides of germanium and the similarly constructed but better known oxides of carbon. Spectroscopic examination of germanium bearing bloods was then made, the results of which seem to confirm the conclusions from chemical analysis and in a measure support the proposed working hypothesis.

From the spectroscopic work the following facts were observed: (a) The absorption spectrum of reduced or venous blood is characteristically different from that of normal blood, and this peculiar spectrum makes its appearance when the germanium-containing blood is reduced *in vitro* by exposing the oxygenated blood to carbon dioxide, reduced pressure or the action of certain oxygen-consuming bacteria. (b) The oxygenated blood containing germanium does not show any noticeable difference from normal oxyhemoglobin. This is in agreement with the analytical results, which indicated that the germanium in the oxygenated blood was to great extent free in the plasma as germanic acid and hence could not modify the normal spectrum of oxyhemoglobin in the cells.

In conclusion, both chemical and spectroscopic analyses lead to the belief that germanium dioxide injected into the system is reduced to the monoxide in the tissues of the animal, probably resulting in the addition of germanous oxide to the hemoglobin of the cells of the venous blood. Subsequent oxygenation of such blood in the lungs then releases the germanium from the cells by converting it to the dioxide, an anhydride of an acid comparable with carbonic acid but non-volatile. The latter appears simply to dissolve in the plasma, where it is forced to remain and return to the tissues with the arterial blood. Here reduction very likely again takes place, reproducing germanous oxide and completing the cycle.

These reactions, if true, plainly indicate that germanium in the blood stream may act as an oxygen carrier, and explain in part the physiological effect which this element produces in the animal system, resulting in erythropoiesis.



# ON THE RELATIONS BETWEEN FERTILITY AND NUTRITION.

## III. THE NORMAL REPRODUCTIVE PERFORMANCE OF THE RAT.

By  
HERBERT McLEAN EVANS  
and  
KATHARINE SCOTT BISHOP.

*From the University of California, and Dairy Division, Bureau of Animal  
Industry, United States Department of Agriculture.\**

1. Introduction.
2. Methods.
3. Correspondence of Oestrous Behavior With the Vaginal Smear.
4. Degree to Which Copulations are Followed by Implantation and the Birth of Young.
5. Average Size of Litters and Behavior of Mother and Young During Lactation.
6. The Numerical Relations Between Ova, Implantations and Living Young.
7. Summary.

### *1. Introduction.*

In order to study upset in either fecundity or fertility, it is essential that we have as a standard, information as to what we may call the normal or average reproductive behavior of the animal investigated and, we believe, of the particular colony used for investigation. Satisfactory data of this kind does not seem to exist for any mammal and it is lacking, for instance, even in the case of domestic animals of great economic importance. Naturally, in order to control variation due to genetic factors, the colony to be studied should be uncontaminated by recent blood admixture and so far as possible be known for many generations preceding the study, in the course of which, as an additional precaution, it should be the invariable rule to employ littermate sisters on experimental and control regimes. To assemble this information for the particular group of animals to which we have devoted our studies, is the object of the present account.

---

\* Work aided by grants from the Committee for Research on Sex Problems of the National Research Council and the California State Dairy Council. The writers wish to express their especial thanks to Mr. C. E. Gray of San Francisco and to Dr. C. W. Larson and Mr. L. A. Rogers of Washington.

## 2. Methods.

A few remarks concerning method appear highly essential since we are convinced that only by the execution of the rather laborious and time-consuming procedure which we have followed can the investigator analyze the cause of a sterility or partial fertility in animals.\* We have already detailed the methods carried out in this laboratory which permit us to recognize the time of oestrus and ovulation in the rat. The mere fact of a reliable method for the recognition of oestrus enables us not only to test sexual responses in animals maintained on experimental diets but to secure the most favorable conditions for obtaining gestation without loss of time. Practically all normal animals will breed during the late pro-oestrous and early oestrous stage, determined by vaginal smears. We are now able to present data on many hundreds of such matings and to demonstrate that an attempt to mate animals at times other than the oestrous period is futile. In this way, young and vigorous stock can be repeatedly tested early in their life cycle before age decreases the perfection of the reproductive mechanism and while resistance to possible intercurrent infection, highest in young animals, is at its best. The animals with which we have experimented have on the incidence of the true epithelial (pro-oestrous) or early cornified cell (oestrous) vaginal smear, been placed with normal young adult, vigorous males for from twelve to twenty-four hours. The males are maintained in individual cages into which the female is introduced. On the following morning the vaginal canal is examined with a small speculum in order to detect the presence of the copulation plug (*bouchon vaginale*). Coincidentally, a microscopic search is made for the detection of sperm, which, if present, are always withdrawn on the small spatula used. The microscopic examination is necessary since the copulation plug has frequently been dislodged, but in all cases in which copulation has occurred sperm may be picked up in the vaginal secretions. On the thirteenth, fourteenth and fifteenth days after a "positive" mating (incidence of the copulation plug or sperm) another vaginal examination is made with the speculum for the presence of blood traces within the vagina, for at this time in normal animals there occurs a slight hemorrhage, or leakage, from the placentae (Long and Evans). Blood is sometimes profuse, but it rarely attains the proportion of what would be designated a hemorrhage and is usually slight in amount, appearing chiefly as a small patch of reddish brown color on the posterior vaginal wall near the cervix. From the existence of this normal "placental leak" we may assure ourselves that implantation and placental function have been established. This is the earliest conclusive sign of gestation known to us in the living animal, and its diagnosis is, of course, of maximum importance in studies of sterility, for by its detection we may assure ourselves that even though some abnormality may exist, the first steps of the reproductive process have been initiated, i. e., insemination, ovulation, fertilization and implantation have been accomplished. We may remark that at all times in the history of the animal, daily vaginal smears are made, and that the "placental leak" is also apparent from the

\* Appendix Table I shows a transcript of a portion of the daily record card for an individual animal.

sudden appearance of erythrocytes among the customary cells of the smear. In the placental deficiency disease which we will describe, placental hemorrhage may occur much earlier than is normally the case.

Littering occurs as a rule on the twenty-second day following copulation. We have made it a practice to isolate the animal on the nineteenth day so that the mother becomes accustomed to her new surroundings and has a maximum chance of littering and suckling her young undisturbed. For four days thereafter in addition to the daily vaginal examination daily weighings are instituted, though at other times these are carried out only at five day intervals. When these procedures are done with care, one is often able to state from this information alone whether, in the absence of young, a resorption or birth has occurred. In the latter case, although the young may have been devoured, the precipitous drop in the mother's weight presents us with a graph very different from that given by the invariably more gradual loss of weight due to resorption. Mother and young are weighed on the day of birth and the young at five day intervals thereafter. The young are weaned on the twenty-first day of life.

### *3. Correspondence of Oestrous Behavior With the Vaginal Smear.*

In their memoir "On The Oestrous Cycle in The Rat" Long and Evans have already stated that during Stage 1, or the pro-oestrous stage, females will not usually mate, but that during the beginning of the following, or oestrous stage (Stage 2) unmistakable signs of heat are usually exhibited. From the arrangement of routine work in our colony, vaginal smears are examined during the morning and animals mated in the afternoon hours. In this way as great an interval of time as eight hours may intervene between diagnosis of the vaginal smear and opportunity for the animal to copulate, and on the average three or four hours intervene between these events. From this it is evident that animals detected as being in the pro-oestrous stage will have reached the oestrous stage or be near it at the time of actual mating of the animals. It is hence true that in studying the inseminations resulting from matings of animals exhibiting some hours earlier the typical pro-oestrous smear, we may be really studying inseminations during the true oestrous stage, and that in studying those from matings following the detection of the oestrous smear, we are but investigating how late after the finding of cornified cells sexual union may take place.

During the last two years many animals have been maintained on a table scrap diet or on the whole wheat-whole milk-casein ration which we have called Standard Diet I.



*Standard Diet I.*  
(*McCollum*)

whole wheat . . . . .	67.5
casein . . . . .	15.0
whole milk powder . . . . .	10.0
sodium chloride . . . . .	1.0
calcium carbonate . . . . .	1.5
milkfat . . . . .	5.0

From such animals maintained on good nutritional regimes we have summarized the findings represented in Table I, from which it is evident that about 77 per cent. of the matings carried out from one to eight hours after the detection of the pro-oestrous smear result in inseminations (as determined by the presence of sperm or copulation plug) and that even 50 per cent. of inseminations occur if mating is attempted following a similar time interval after the recognition of the true oestrous smear. This represents a considerable reduction in the incidence of sexual union in accordance with the time chosen for the pairing or mating of animals. And yet it is truly surprising that this amount of success is attained when we view the fact that no instances of insemination have been secured by us when animals were mated in the dioestrous interval.

TABLE I.

Showing the Number of Instances of Insemination When Animals Were Mated Within Eight Hours After the Diagnosis of the Pro-oestrous and Oestrous Stages Respectively

Vaginal Smear	Total Number of Matings	Total Number of Inseminations	Percentage of Inseminations
Proestrous.....	659	507	77%
Oestrous.....	425	217	51%

The above table cannot be taken as an index of the success which would attend mating normal animals immediately on the detection of the pro-oestrous smear, for it is our experience that most of such animals mate. The data, consequently, cannot convey information as to the degree to which coitus is accepted by normal oestrous animals, but only information upon the relative advantage of mating animals as soon as possible after the incidence of the preliminary oestrous changes in the vaginal smear.\*

\* There undoubtedly occurs a low proportion of instances of inactivity on the part of males, even though these be chosen as carefully as is possible and allowed recuperation from sexual activity.

No inconsiderable interest attaches to the fact that there are also somewhat greater chances of an insemination leading to pregnancy when copulation is permitted earlier in the cycle. In a relatively small group of rats (one hundred and sixty-eight individuals) it was possible for us to follow the correspondence between mating, insemination and placentation when these events transpired after a mating following the detection of the pro-oestrous and oestrous smears respectively.

TABLE II.

Showing the Number of Instances of the Establishment of Placentae as Shown by the Fourteenth Day "Placental Sign," After Mating Following the Pro-oestrous and Oestrous Stages Respectively In a Group of 168 Rats

Vaginal Smear Four Hours Previous to Mating	Total Number of Matings	Total Number of Inseminations	Total Num- ber of Instances of Placental Sign	Percentage of Inseminations Giving Implantation
Pro-oestrous . . . .	311	249	216	86.8%
Oestrous . . . . .	179	87	69	79.3%

#### 4. *Degree to Which Copulations Are Followed by Implantation and the Birth of Young.*

Although the term fertility has always been used to denote the capacity of an animal to produce living young, the term has not been qualified by precise measurements of this capacity. It is obvious that the degree of fertility may be conditioned by all of the factors which we have enumerated as entering into the physiology of reproduction and that the degree of fertility itself may be stated either in terms of the average number of young produced or by the extent to which sexual congress may be predicted as leading to the birth of living young. We shall speak of the fertility ratio, or fertility per cent., as the ratio existing between the number of positive matings (as detected by finding sperm or the copulation plug) and the number of litters born, expressed as per cent. with the number of inseminations, or positive matings, as 100. This ratio may be computed for a group of rats or for the individual. In the latter case it is, of course, the individual fertility per cent. and must be arrived at by testing the individual without reference to the average for the class.\* Space and time have forbidden us the advantage of invariable test matings in our

\* The "mating fertility per cent." studied by Reynolds and Macomber does not enter into our decision since we are in all cases employing males on adequate diet and presumably of normal vigor.

experimental work, i.e., mating a female of known fertility with the same male and at the same approximate time at which the experimental female is mated. We have, however, made a series of at least four positive and unsuccessful matings, each with a different male, before pronouncing a female sterile. Furthermore, we have kept records of the males used in all experimental matings so that repeated failure to conceive or to breed has been traced to the male, if the latter is at fault. On account of these procedures, it is likely that only a small proportion of the unsuccessful matings which we shall report can be explained by lack of vigor on the part of the male even though the males employed may have been conceivably at some periods overtaxed.

Even under unusually good conditions, only approximately 80 per cent. of inseminations may lead to the birth of young (group fertility per cent.). When animals have been maintained on adequate nutritional regimes and submitted to daily study so that mating is attempted only at the appropriate time in the vaginal smear and the acceptance or rejection of coitus known by the next daily examination and, furthermore, when there is known whether or not we have an establishment of the placenta, by the presence or absence of the fourteenth day "placental sign," data such as is presented in Table III may be secured.

TABLE III.

Summary of the Reproductive Performance of 168 Animals Maintained on Satisfactory Nutritive Regimes and Submitted Each to from One to Six Inseminations. (The Detailed Histories Are Given in Appendix Tables II, III and IV)

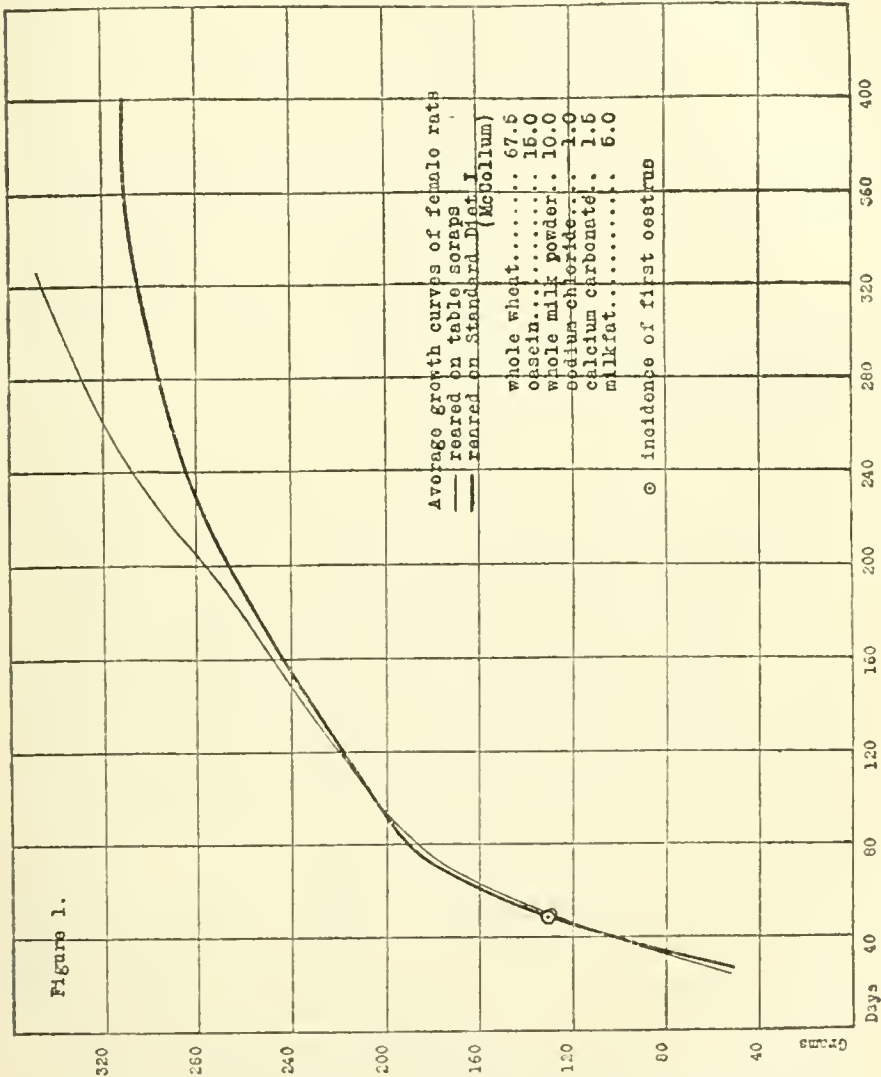
DIET	Age in Months at First Mating	Number of Individuals	Number of Positive Matings as Determined by Plug or Sperm	Placental Sign Found	Number of Litters Born	Group Fertility Per Cent	Implantation Per Cent	Placental Index
Table Scraps . . . . .	4-8	37	85	70	70	82%	82%	100%
Standard Diet I (reared upon table scraps) . .	4-10	54	85	80	76	89%	94%	95%
Standard Diet I (reared on this diet) . . . . .	3-12	77	166	138	135	81%	83%	98%
TOTAL . . . . .	3-12	168	336	288	281	84%	86%	97.5%

It is to be emphasized that from such data all instances of proven or suspected infection of the generative tract should be rigorously excluded. The investigator of sex phenomena in the rat who remains unaware of such disease, will confuse a deficiency which has been experimentally produced with that imposed by an infectious venereal disorder. In this, of course, he will err more grievously than do students of growth who ignore the notoriously frequent pulmonary or middle ear infections of the rat, for in the early stages of life the latter infections may not prevent growth, but the venereal disease to which we refer almost invariably itself confers sterility upon its victim. The infectious sterility is, fortunately, betrayed by the fact that in its presence, implantation is impossible. In such instances copulation is never followed by the placental sign; normally, two such successive matings are rare. When in the case of one individual four or more successful or positive matings fail to yield the placental sign, we have discarded these and all subsequent portions of the history of the case, assuming infection. Many such cases have been autopsied. Half of them show at once outspoken macroscopic evidence of disease of the oviducts—pyosalpinx—and the remainder will probably give microscopic evidence of the presence of the same infection. The uterus may often seem normal or be but slightly distended and hyperaemic or with yellowish or orange pigmentation. The ovaries are usually normal. Frequently occlusion of the oviducts leads to an accumulation of fluid within the *bursa ovarica*, which is thus slightly or enormously distended. The fluid may be clear, bloody, or cloudy with pus. One or more loops of the oviduct will be found distended, frequently with thick, yellow pus; the subacute stages of the disease, however, may give a slighter distension of these folds and fluid which to the naked eye seems almost clear. Students of the rat have often complained of the great scourge occasioned by pulmonary disorder. We would warn students of sexual phenomena that we have frequently found almost half of a miscellaneous lot of stock animals infected with oviducal disease, and it may be widely prevalent even in carefully selected groups where maximum attention has been given to isolation, to cleanliness and to other care as well as to nutrition. As an example of the worthlessness of data secured without control as to disease, we may state that while we were working with our general colony of animals, out of a total of 1079 positive matings only 631 gave



the fourteenth day placental sign and but 534 records of littering were made. This would give a fertility per cent. of but 50, only of interest to us perhaps as the lowest average secured from uncontrolled and unselected cases. It is to be especially emphasized that the disease does not interfere with the ovulation rhythm or with excellent growth or maintenance of body weight. The infection was noted by Long and Evans and first called their attention to a characteristic prolongation of the oestrous cycle which follows infertile positive matings. The cycle is prolonged from one of four days to ten or even seventeen days before another oestrus appears, a phenomenon, as Long and Evans have shown, associated with preliminary pregnancy changes and justifying the term "pseudopregnancy." They have established the same picture by breeding normal females with vasectomized males and by mechanical stimulation of the cervical canal and hence feel the condition stands in some relation to the copulation mechanism. It is not impossible that the phenomenon is a necessary part of gestation and that possibly part of the low, but constant, percentage of failures to implant in normal animals may be attributed to failure of this mechanism. We have examined the times of recurrence of oestrus in such cases of failure to conceive in our normal animals and in over one-third of these instances (themselves rather rare) the oestrous cycle was, in fact, not appreciably prolonged. Attention should therefore be given to such an explanation. However, it is apparent that there still remains in normal animals the problem of positive matings which normally prolong the oestrous cycle but do not result in a positive placental sign. These may occur between instances of the birth of healthy litters so that normality should be assumed. In such instances, with normality of what we have called the copulatory mechanism in starting the gestation, it would appear that we must look upon the failure as due to lack of germ cell vigor on the part of either male or female or to interference with the uterine implantation mechanism. The latter explanation appears to us doubtful inasmuch as in the specific placental deficiency disease which we shall describe and in which the uterus is gravely impaired, placentation is successfully accomplished. We hope that we may be able in the future to complete the analysis of this problem. In the meantime we may state that under the usual conditions prevailing in our work and with the exclusion of the factor of disease, in from eighty to ninety per

cent of the instances, any single copulation results in a full term, normal pregnancy.\* The sex histories from which Table III has been compiled, are given in full in Appendix Tables II, III and IV and the growth curve of two of the groups is given in Figure 1.



\* The only figures in the literature with which these data may be compared are those reported by King (80 per cent.), Reynolds and Macomber (65 per cent. to 96 per cent.) and by Long and Evans (88 per cent.).



If the histories from which Table III has been compiled are studied for the individual fertility exhibited by the rats in each of the three nutritive groups, the results may be tabulated as in Table IV. This table shows essentially that in a group of animals maintained in good nutrition and where in each individual we have studied merely the results of from one to six copulations, at least 75 per cent. of the animals show "perfect" behavior, i.e., each and every insemination is followed by the birth of a litter.

TABLE IV.

Showing the Individual Fertility of the Animals Used in the Compilation of Table III

DIET	Number of Rats	ANIMALS EXHIBITING 100% FERTILITY		ANIMALS EXHIBITING NO FERTILITY	
		Number	Per Cent	Number	Per Cent
Table Scraps.....	37	31	84%	0	0%
Standard Diet I (reared upon table scraps)...	54	47	87%	0	0%
Standard Diet I (reared upon this diet).....	77	58	75%	2	3%
TOTAL.....	168	136	81%	2	1%

#### 5. *Average Size of Litters and Behavior of Mother and Young During Lactation.*

The average sized litter in our colony consists of seven. The figures happen to be in agreement with the extensive data collected by King and Stotsenberg at the Wistar Institute. In 1920 Long and Evans reported this figure from the analysis of somewhat over six hundred litters in our colony. Their results are expressed in a column of Table V. Our total data represent the analysis of 1345 litters cast during the last five years and arranged in lots in accordance with the time when the observations were made or with the nutrition of the group.

In order that the performance of lactation could be judged by having animals as nearly as possible with the same lactation task, we have destroyed as many of the young in larger litters as would leave the number at six. It was felt that this or a smaller number which might happen to be born should be successfully suckled or, at any rate, would give a good index of the extent to which animals on good nutritional regimes can carry out the function of lactation. Table VII presents these data.

TABLE V.

Showing Number of Young per Litter of Animals Maintained On Various Dietary Regimes

DIET	Total Number of Litters	Total Number of Young	Average Number of Young per Litter	MODE
Table Scraps — Long-Evans Stock, 1917-1920	625	4371	6.99	7
Table Scraps — Stock, 1920-1922.....	501	3649	7.3	7
Table Scraps — 1920-1922 (Special Group).....	58	372	6.4	7
Standard Diet I (reared upon table scraps).....	68	470	6.9	7
Standard Diet I (reared on this diet).....	93	545	5.9	6
TOTAL.....	1345	9407	6.99	7.0

TABLE VI.

Instances of Litters Grouped According to Number of Young In Litter

Number in Litter	Table Scraps Long-Evans 1917-1920	Table Scraps Stock 1920-1922	Table Scraps 1920-1922 (Special Group)	Standard Diet I (Reared Upon Table Scraps)	Standard Diet I (Reared Upon This Diet)	Total
1.....	1	3	1	1	2	8
2.....	8	18	1	2	10	39
3.....	27	15	4	1	5	52
4.....	58	27	8	5	8	106
5.....	70	45	8	7	11	141
6.....	110	72	1	10	19	212
7.....	114	81	18	16	14	243
8.....	105	75	9	10	13	212
9.....	68	72	3	10	6	159
10.....	43	49	2	2	4	100
11.....	14	29	2	3	1	49
12.....	12	11	1	1	0	25
13.....	3	2	0	0	0	5
14.....	1	2	0	0	0	3
15.....	0	0	0	0	0	0

The mother and her young are weighed within twenty-four hours after parturition and on the twenty-first day of life, when weaning is instituted. We have consequently accumulated a

TABLE VII.

Proportion of Young Which are Suckled When Mother is Permitted to Suckle Six or Less Young

DIET	Total Number of Young to Be Suckled	Number of Young Weaned	Per Cent
Table Scraps.....	267	213	80%
Standard Diet I (reared upon table scraps).....	191	126	66%
Standard Diet I (reared upon this diet).....	410	336	82%
TOTAL.....	868	675	78%

considerable body of data on the gain or loss in the mother's weight and on the growth of the young. As regards the birth weight and weaning weight of the young, Table VIII shows that without reference to sex they grow on the average from a weight of six to one of forty-three grams, an increase of 724 per cent.\*

TABLE VIII.

Average Weight of Young of Rats Which Had Been Reared on Various Diets—at Birth and at Weaning

DIET	Total Number of Young Born	Average Weight of Young at Birth (in Grams)	Number of Young Weaned	Average Weight of Young at Weaning (21st Day) (in Grams)	Percentage Gain
Table Scraps .Stock (Liquid Standard Diet I).....	1443	5.9	849	43.4	736%
Table Scraps Only (Special Group).....	366	6.0	220	40.7	678%
Standard Diet I (reared upon table scraps)...	217	5.8	123	41.3	712%
Standard Diet I (reared upon this diet).....	537	5.8	332	42.6	734%
TOTAL.....	2563	5.9	1524	42.7	724%

\* The first weight (5.9 grams) is not actually an average birth weight but the average weight towards the end of the first day of life and after suckling has, of course, filled with milk the alimentary tract of the young.

The slightest gain for the young in any of the above groups was in the case of animals held exclusively upon table scraps, where the weaning weight was nearer forty-one than forty-three grams. These were the only mothers from whom was withheld during lactation an accessory liquid diet containing milk, (Standard Diet I), which evidently has specific value in aiding lactation.

It will be of value to record the extent to which litters of normal animals are successfully suckled. Under the best of nutritional conditions, instances are encountered in which all of the litter are devoured by the mother, abandoned by her or for unknown causes eventually die before the day of weaning. In Table IX we have summarized our experience on the extent to which at least some members of a litter are suckled throughout the lactation period of twenty-one days.

TABLE IX.

Proportion of Litters in Which Some Young are Suckled

DIET	Total Number of Litters	Litters Weaned (21st Day)	Per Cent
Table Scraps.....	58	49	84%
Standard Diet I (reared upon table scraps).....	67	59	88%
Standard Diet I (reared upon this diet).....	89	76	85%
TOTAL.....	214	184	86%

The mother's loss or gain in weight during lactation is shown in Table X, where it will be noted that there is usually an actual gain of from 5 per cent. to 8 per cent. of the body weight during lactation. Only in the group from which milk was withheld during lactation (table scraps only) was there a loss (-3 per cent.).

#### 6. Numerical Relations Between Ova, Implantations, and Living Young.

While these data may be deemed sufficient to serve as a standard with which we may compare the fertility of groups of the closely related animals in our own colony, yet they do not give sufficient information as to the degree to which the average number of germ cells liberated at an ovulation are represented as full term healthy young. It is conceivable and of course, likely

TABLE X.

Lactation Performance. Table Showing Weights of Mother at Littering and at Weaning

DIET	Number of Mothers	Average Weight at Littering (In Grams)	Average Weight at Weaning (In Grams)	Average Gain in Weight (In Grams)	Percentage Gain in Weight
Table Scraps Stock (Liquid Standard Diet I) . . . . .	238	263.9	285.5	+21.6	+8.2%
Table Scraps Only (special group) . .	44	282.6	273.6	- 9.0	-3.2%
Standard Diet I (reared upon table scraps) . . . . .	54	240.9	260.5	+19.6	+8.1%
Standard Diet I (reared upon this diet) . . . . .	68	266.8	280.7	+13.9	+5.2%

that upset to fertility could still be grave and yet permit the birth of a depleted litter of living young. The perfection of the steps in the reproductive mechanism can only be analyzed by having recourse to autopsies performed late in the course of gestation. Under such conditions the only corpora lutea of appreciable size present in the ovary are the corpora of pregnancy and represent therefore the number of ova shed at the last ovulation. Animals killed on the nineteenth day of gestation have been examined with reference to the number of corpora in each ovary and the number of normal or abnormal implantation sites in each uterine horn. Abnormal or resorbed implantations are always easily identified and cannot be confused with the placental sites of previous pregnancies since even in two immediately successive pregnancies the sites of the former pregnancy are only minute, pigmented scars at the mesometrial border of the uterus, in comparison with the larger amount of mesometrial tissue representing the resorption. The latter always persist throughout the twenty-two days of gestation, although the foetus may have disappeared and foetal tissue may be indistinguishable macroscopically. Table XI shows the numerical relations which we have found to exist between the number of ova shed (as determined by the number of corpora lutea present) and the number of implantations. It shows that approximately 70 per cent. of

the ova matured at the conception ovulation are represented by implantations and that normally about one in ten of the actual implantations is represented by resorptions.

King has recently made a valuable analysis of birth mortality in the rat, comparing it with that obtaining in the case of man. From a large experience, she estimates that in normal cases there are about 2 per cent. of young born dead. It may consequently be of interest for us to place on record statistics secured from our three groups of animals, even though the numbers involved are very limited.

King has stated certain grounds for believing that the mortality at parturition is due to defective foetal nutrition. Studies such as ours would seem calculated to throw light upon this point, and we may hope to be able to handle the question at a future date.

TABLE XI.

Showing the Numerical Relations Between Ova Shed (Corpora Lutea) and Those Implanted Either Successfully (Living Foetuses) or Unsuccessfully (Resorptions)

DIET	Number of Cases	Number of Corpora	Number of Living Foetuses	Number of Resorptions	Average Number of Corpora per Case	Average Number of Foetuses per Case	Average Number of Resorptions per Case	Percentage of Ova Implanted	Percentage of Implantations Resorbed
Table Scraps.....	10	91	71	2	9.1	7.1	.2	80%	2.7%
Standard Diet I (reared upon table scraps).....	6	55	26	3	9.1	4.3	.5	53%	10.3%
Standard Diet I (reared upon this diet).....	36	365	226	27	10.1	6.3	.75	69%	10.7%
TOTAL.....	52	511	323	32	9.8	6.2	.615	69%	9%
						6.82			
						Total implantations			

In the meanwhile it is certain that the numbers with which we have dealt while inadequate in a statistical sense, portray the limits of parturition mortality on "adequate" diets sufficiently to serve as a control in studies of the extent to which this phenomenon may be increased by defective diets.



TABLE XII.  
Occurrence of Young Born Dead

DIET	Number of Mothers	Total Number of Young	Number Born Dead	Percent of Young Born Dead
Table Scraps . . . . .	37	372	7	1.9%
Standard Diet I (reared on table scraps) . . . . .	54	470	14	3.0%
Standard Diet I (reared upon this diet) . . . . .	77	545	37	6.8%
TOTAL . . . . .	168	1387	58	4.2%

### 7. Summary.

1. Attention has been called to the necessity of securing data on the normal reproductive performance of the particular group of animals used by an investigator for experimental studies in fertility and sterility as these may be influenced by nutritive regimes. These are furnished for a colony in which such studies are in progress.

2. In reproduction tests, full advantage should be taken of our knowledge of the oestrous cycle. Animals mate only at this time. If animals are tested at this time, the acceptance of coitus can be positively confirmed by the microscopic detection of residual sperm. Thus within a few months and before age decreases the perfection of the reproductive mechanism and while resistance to infection is highest, positive rather than presumptive evidence of infertility or fertility can be secured by following the fate of positive matings.

3. Only matings attempted promptly after the occurrence of the prooestrous stage lead in a high proportion of cases to the consummation of coitus. This is secured in only half the cases mated later in the oestrous stage. In the latter cases, even if positive mating results, there is somewhat less chance of pregnancy resulting (80 per cent. instead of 87 per cent.).

4. In normal animals of good vigor, at least 80 per cent. of inseminations lead to birth of young.

5. In almost all cases the establishment of the placenta is followed by the birth of young (97.5 per cent.). Complete placental failure is very rare in normal animals.

6. In the short time interval covered by the experiment, somewhat over 80 per cent. of normal animals became pregnant after every single insemination.

7. On the average, seven young are born per litter and this is also the most frequently occurring size of litter.

8. Eighty-six per cent. of all litters born were represented by one or more young successfully suckled and where the litters were restricted to six or fewer young, almost 80 per cent. of the young permitted to suckle were successfully suckled.

9. If mothers are given the Standard Diet I in liquid form during the twenty-one days of suckling, the young, on the average, increase their weight from 6 to 43 grams or over 700 per cent.

10. Mothers, if given liquid Standard Diet I during lactation, gain from 5 per cent. to 8 per cent. in weight in spite of the handicap of suckling young.

11. On the average, 9 or 10 eggs are matured at each ovulation with an average of only 6 or 7 young born so that only approximately two-thirds of the sex cells are represented in young; almost all of this failure is in the steps preceding implantation since there are, on the average, 7 implantations per gestation; the failure is probably attributable to lack of fertilization or to poor germ cell vigor rather than to failure of the uterus to respond to implantation. Varying somewhat, from 3 per cent. to 11 per cent. of all the implantations observed in 52 cases of normal pregnancy (355 implantations) were defective and partly resorbed. Although equal numbers of cases were not studied, fewest of these happened to occur on the miscellaneous table scrap ration, most on the whole wheat-whole milk-casein ration (Standard Diet I). There is a similar increase of parturition mortality in animals held on the latter ration.

#### BIBLIOGRAPHY.

- Donaldson, H. H. *The Rat. Memoirs of the Wistar Inst. of Anat. and Biol.*, No. 6, Philadelphia, 1915, 278 pp., 8°.
- Evans, H. M. and Bishop, K. Scott. On the relations between fertility and nutrition. I. The ovulation rhythm in the rat on a standard nutritional regime. *J. Met. Res.*, 1, 1922, 319.
- Evans, H. M. and Bishop, K. Scott. On the relations between fertility and nutrition. II. The ovulation rhythm in the rat on inadequate nutritional regimes. *Ibid.*, 335.

- King, H. D. The relation of age to fertility in the rat. *Anat. Record*, 11, 1916, 269.
- King, H. D. Studies on inbreeding. II. The effects of inbreeding on the fertility and the constitutional vigor of the albino rat. *J. Exper. Zool.*, 26, 1918, 335.
- King, H. D. A comparative study of the birth and mortality in the albino rat and in man. *Anat. Record.*, 20, 1921, 321.
- Long, J. A. and Evans, H. M. The oestrous cycle in the rat and its associated phenomena. *Memoirs of the Univ. of Calif.*, VI, Univ. of Calif. Press, Berkeley, 1922, 148 pp., 11 pl., 4°.

## APPENDIX TABLE I.

Example of the daily record kept in the case of every animal used for experimentation: History of the female *W 8899*.

Born: Aug. 16, 1922; Parents: B 00; Littermates:

BH 8900, B 8901, B 8902; Weighing Group IV.

DIET: Standard Diet II with the addition of dried yeast daily in approximate doses of .6 gram.

Mother reared on table scraps and liquid Standard Diet I during lactation.

Sept. 6	Weaned and placed on Standard Diet II	39 gms.
8	Started yeast feeding	
9	Vaginal orifice closed	
10	" " "	12 gms.
11	" " "	
12	" " "	
13	" " "	
14	" " "	
15	" " "	53 gms.
16	" " "	
17	" " "	
18	" " "	
19	" " "	
20	" " "	72 gms.
21	" " "	
22	" " "	
23	" " "	
24	" " "	
25	" " "	84 gms.
(Birth to opening and first oestrus 44 days)	26 " " "	
	27 " " "	
	28 " " "	
	29 Vaginal orifice open—Cornified	
	30 Leucocytes and epithelial cells	101 gms.
Oct. 1	" " " "	
2	" " " "	
3	" " " "	
4	" " " "	
(9) 5	" " " "	118 gms.
6	" " " "	
7	" " " "	
— 8	" " " "	
	9 Cornified cells only	
	10 " " "	128 gms.
	11 Leucocytes and epithelial cells	
(5) 12	" " " "	
— 13	Epithelial cells only	
	14 Epithelial and cornified cells	
	15 Cornified cells only	141 gms.
(5) 16	Epithelial, cornified cells and leucocytes	
	17 Leucocytes and epithelial cells	
— 18	Epithelial and cornified cells	
	19 Cornified cells only	
(4) 20	Epithelial, cornified cells and leucocytes	150 gms.
21	" " " "	
— 22	Epithelial cells only	
	23 Cornified cells only	
(4) 24	Epithelial, cornified cells and leucocytes	
	25 Leucocytes and epithelial cells	158 gms.
— 26	Epithelial cells only; placed with male 459.	
27	No plug; sperm found	

APPENDIX TABLE I.—*Continued*

	28	Cornified cells and leucocytes				
	29	Leucocytes and epithelial cells				
	30	"	"	"	"	168 gms.
	31	"	"	"	"	
Nov.	1	"	"	"	"	
	2	"	"	"	"	
	3	"	"	"	"	
	4	"	"	"	"	170 gms.
	5	"	"	"	"	
	6	"	"	"	"	
	7	"	"	"	"	
	8	"	"	"	"	
	9	"	"	"	"	RBC
	10	"	"	"	"	RBC
	11	"	"	"	"	RBC
	12	"	"	"	"	RBC
	13	"	"	"	"	
	14	"	"	"	"	194 gms.
(22)	15	Isolate—cage 7				196 gms.
	16	No litter; RBC; Leucocytes and epithelial cells				196 gms.
	17	"	"	"	"	190 gms.
	—18	Returned. Epithelial cells only				188 gms.

APPENDIX TABLE II  
Reproductive performance of rats reared on a diet of table scraps

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Litter	NUMBER OF YOUNG		AVERAGE WGT. (in gms.)		WEIGHT OF MOTHER (in gms.)		AUTOPSY RECORD				Average Weight of Foetuses in Corpus	Notes
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora lutea	Living foetuses	Resorptions			
BH 5011	8 mos.	W 5699	•	•	•	?	5	not weighed	45.6	258	235						
		G 5718	•	•	•	11	6	34.0	38.4	381	366						
		B 5717	•	•	•												
BH 5013	8 mos.	W 5872	•	•	•	3	3	6.7	59.3	262	256						Autopsied 19th day of gestation
		W 4802	•	•	•	7	0	6.7		306							Litter lived 2 days only.
		W 27	•	•	•	8	6	6.1	36.3	306	272						Autopsied 19th day of gestation.
		GH 55	•	•	•												
B 5023	9 mos.	B 5669	•	•	•	9	5	5.3	41.7	270	270						
		GH 5865	•	•	•	7	5	6.6	38.4	302	316						
		GH 10	•	•	•												
W 5032	8 mos.	B 5698	•	•	•	3	3	6	49.3	240	241						Autopsied 19th day of gestation.
		BH 80	•	•	•	4	2	6	37.5	272	256						
		GH 54	•	•	•												
W 5053	8 mos.	BH 5719	•	•	•	5	3	4	35.3	230	274						Autopsied 14th day of gestation.
W 5065	8 mos.	B 5669	•	•	•	8	5	5.7	48.6	222	197						Discarded.
G 5087	8 mos.	BH 6554	•	•	•	4	0	4.2		226							4 more matings without placental sign.
			•	•	•												Autopsied. Infected.
G 5004	7 mos.	W 5236	•	•	•	7	6	5.4	39.1	237	224						4 more matings without placental sign.
			•	•	•												Autopsied. Possibility of infection.
W 5008	7 mos.	G 5716	•	•	•	7	0	6	48.5	274	360						Discarded.
W 5141	7.5 mos.	W 5717	•	•	•	?	?	not weighed	48.5	344							
		W 5236	•	•	•	?	?	not weighed	283	280	283						
			•	•	•												
		G 5720	•	•	•	7 (1 dead)	3	4.8	33	332	326						
BH 5112	7 mos.	W 5717	•	•	•	7	?	6		269							2 more matings without placental sign.
			•	•	•												Autopsied. Infected.
G 5151	7 mos.	W 4802	•	•	•	3	2	6.7	47.5	288	313						5 more matings without placental sign.
B 5164	8 mos.	W 5236	•	•	•	?	5	not weighed	41.2	306	300						Autopsied. Infected.
			•	•	•												8 more matings without placental sign.
		W 5401	•	•	•	11	6	6.5	33.1	372	321						Autopsied. Infected.





GH 5626	5 mos.	B 46 BH 46 W 68 GH 5855 BH 5160 BH 00 W 5426	• • • • • • •	0 0 0 0 0 0 0	• • • • • • •	7 7 • • • • •	6 5 • • • • •	6.4 6.3 • • • • •	31.1 35.4 • • • • •	268 340 • • • • •	266 318 not weighed 184 227 236 241 228	5 5 • • • • •	6 6 • • • • •	5 5 • • • • •	8 8 • • • • •	5 5 • • • • •	0 0 • • • • •	4 4 • • • • •	Autopsied 19th day of gestation.  Autopsied 19th day of gestation. 3 more matings without placental sign. Autopsied. Possibility of infection. 3 more matings. Autopsied. Infected  Autopsied 2 days after mating Normal. Discarded. 3 more matings without placental sign. Autopsied. Infected.  Autopsied 20th day of gestation  4 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.  Autopsied 19th day of gestation.  Autopsied 19th day of gestation. Lung infection. 2 more matings. Autopsied. Normal.
B 5639	5 mos.	G 5230	•	•	•	7	?	6.1	not weighed 18.6	249	249	5	6	5	5	0	0	2	4
B 5640	5 mos.	G 5718	•	•	•	7	7	5.1	180	180	184	•	•	•	•	•	•	•	•
W 5658	5 mos.	W 5235 W 5236	• •	• •	• •	7 9	3 ?	2.8 3.5	43 not weighed 34	227 265	236 241	• •	• •	• •	• •	• •	• •	• •	• •
W 5732	4 mos.	B 5698	•	•	•	•	5	4	0	261	228	•	•	•	•	•	•	•	•
W 5737	4 mos.	G 5720 BH 6551	• •	• •	• •	8 2	6 (1 dead)	5.6 5	45.3	302 370	288	•	•	•	•	•	•	•	•
W 5741	4 mos.	BH 5719 BH 82 BH 5460	• • •	• • •	• • •	• 5 •	4 5 •	6.5 6.4	15.7 40	284 346	274 332	•	•	•	•	•	•	•	•
W 5743	4 mos.	W 4699 W 5401	• •	• •	• •	3 10	3 ?	8 6	57.3	340 352	320	•	•	•	•	•	•	•	•
W 5745	5 mos.	G 5716 B 5659 B 5717 W 4699 W 27	• • • • •	• • • • •	• • • • •	• • • • •	8 6 6 6 6	6 6 6 6 6	33.6	273	241	•	•	•	•	•	•	•	•
B 5748	5 mos.	B 5669 W 4872 W 605	• • •	• • •	• • •	5 7 •	4 5 •	6.2 6.6	51.5 47.4	278 349	266 295	•	•	•	•	•	•	•	•
B 5749	5 mos.	GH 5634 GH 29	• •	• •	• •	8 4	0 0	5.8 6.0	360 392	360 392	•	•	•	•	•	•	•	•	•

Totals: 37 mts.  
85 positive matings.  
70 findings of placental sign.  
70 litters.



W 5003	4 mos.	W 4686	•	•	•	7	?	•	•	207	251					Transferred to Standard Diet I at 5 mos. (Given greens also). Transferred to Standard Diet I at 5 mos. (Given greens also).
B 5004	4 mos.	W 4686	•	•	•	6	?	•	•	200	246					
GH 5005	4 mos.	G 7	•	•	•	•	•	•	•	193	235					Transferred to Standard Diet I at 5 mos. (Given greens also).
GH 5008	4 mos.	GH 4600	•	•	•	8	?	•	•	166	192					Transferred to Standard Diet I at 5 mos. (Given greens also).
B 5009	4 mos.	W 4754	•	•	•	10	?	•	•	267	304					Transferred to Standard Diet I at 4 mos.
BH 5012	8 mos.	B 5716	•	•	•	9	6	6	4	47.3	246	262	285			Autopsied 19th day of gestation. Transferred to Standard Diet I at 4 mos. (Given greens also).
W 5014	9 mos.	G 4707	•	•	•	7	6	7	4	47.1	265					
W 5015	4 mos.	BH 5710	•	•	•	4	4	5	5	41.5	280	236				Transferred to Standard Diet I at 4 mos. (Given greens also).
W 5016	1 mos.	W 5038	•	•	•	8	0	6	2	330	230					Transferred to Standard Diet I at 4 mos. (Given greens also).
W 5017	4 mos.	BH 5081	•	•	•	0	?	•	•	172	185					Transferred to Standard Diet I at 4 mos. (Given greens also).
W 5018	4 mos.	BH 55	•	•	•	9	?	•	•	180	224					Transferred to Standard Diet I at 4 mos. (Given greens also).
W 5019	4 mos.	BH 55	•	•	•	7	?	•	•	170	218					Transferred to Standard Diet I at 1 mos. (Given greens also).
W 5024	9 mos.	W 1000	•	•	•	12	?	•	•	216	235					Transferred to Standard Diet I at 1 mos. (Given greens also).
GH 5029	9 mos.	BH 6554	•	•	•	7	0	5	4	45.1	322	332				Transferred to Standard Diet I at 4 mos. (Given greens also).
W 5031	9 mos.	W 4699	•	•	•	7	6	5	4	38.6	226	260				Autopsied 14th day of gestation. Transferred to Standard Diet I at 4 mos. Lung infection. Discarded.
B 5061	8.5 mos.	W 5235	•	•	•	7	3	6	1	54.6	242	264				Transferred to Standard Diet I at 1 mos. Sickly. Discarded.
W 5080	8 mos.	W 5236	•	•	•	5	?	5	2	not weighed	250	not weighed				Transferred to Standard Diet I at 3 mos. Lung infection. Discarded.
W 5086	8 mos.	W 5717	•	•	•	9	8	5	6	26.7	262	272				Transferred to Standard Diet I at 3 mos. Discarded.
G 5089	7 mos.	G 5718	•	•	•	6	5	6	3	35.4	216	246				Transferred to Standard Diet I at 3 mos. 2 more matings without placental sign. Autopsied. Infected.
BH 5093	7 mos.	G 5718	•	•	•	2	0	5	5	195	219					Transferred to Standard Diet I at 3 mos. Sickly. Discarded.
W 5100	8 mos.	BH 400	•	•	•	8	5	4	0	46.0	203	219				Transferred to Standard Diet I at 3 mos. Lung infection. Discarded.
G 5101	8 mos.	G 5716	•	•	•	4	0	6	5	210	266	286				Transferred to Standard Diet I at 3 mos. Lung infection. Discarded.
W 5114	8 mos.	W 1699	•	•	•	7	6	6	3	41.3	361					Transferred to Standard Diet I at 3 mos. (3 dead)
		4872	•	•	•	5	0	4	8							

APPENDIX TABLE III—Continued

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Litter	NUMBER OF YOUNG		AVERAGE WGT. OF YOUNG (in gms.)		WEIGHT OF MOTHER (in gms.)		Autopsy Record				Fetuses in Grms.	NOTES
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora lutea	Living foetuses	Resorptions	Average Weight of		
												R	L	R	L		
W 5115	8 mos.	BH 4704	*	*	*	7	5	6.4	45.6	370	372						2 more matings without placental sign. Autopsied. Infected. Transferred to Standard Diet I at 3 mos.
BH 5129	8 mos.	W 4872	*	*	*	6	6	6.1	47.0	276	272						Lung infection. Discarded. Transferred to Standard Diet I at 3 mos.
		G 4707	*	*	*	7	6	6.4	34.0	254	201						
		B 5518	*	*	*	10	6	6.2	262	262							
		BH 4709	*	*	*	5	0	3.8	206	206							Transferred to Standard Diet I at 3 mos.
		G 45	*	*	*	(1 dead)											
		G 60	*	*	*	0											
GH 5143	8 mos.	W 4872	*	*	*	2	2	6.0	38.0	218	231	?	2	0	0		Autopsied 12th day of gestation. Transferred to Standard Diet I at 3 mos.
		W 1000	*	*	*	7	3	not weighed	40.6	238	250						
GH 5144	8 mos.	W 1000	*	*	*	5	?	5.6	not weighed	234	206	8	3	1	1	0	Autopsied 19th day of gestation. Transferred to Standard Diet I at 3 mos.
		W 4872	*	*	*	6	3	6.6	39.0	275	307	6	3	1	1	0	
		GH 60	*	*	*												
		W 5236	*	*	*												
B 5152	8 mos.	W 1000	*	*	*	5	4	6.8	47.5	262	314					not wd.	Autopsied 19th day of gestation.
B 5103	10 mos.	BH 4709	*	*	*	7	5	6.3	31.0	254	263						Transferred to Standard Diet I at 3 mos. Autopsied after 5 more matings without placental sign. Infected.
W 5226	8 mos.	W 5717	*	*	*	3	3	6.0	51.3	250	255						Autopsied after 2 more matings without placental sign. Possibility of infection.
		B 51	*	*	*	7	6	6.1	43.1	251	243						Transferred to Standard Diet I at 3 mos.
W 5228	8 mos.	G 5230	*	*	*	8	6	5.5	39.8	216	212	4	4	3	0	0	Autopsied 19th day of gestation.
W 5231	8 mos.	G 5718	*	*	*	6	6	5.1	208	208							
		W 4802	*	*	*	4	0	5.7	205	205							
		BH 5860	*	*	*	6	0	6.2	233	233							
		W 61	*	*	*	7	6	6.3	35.8	228	249						Lung infection. Discarded. Transferred to Standard Diet I at 3 mos.
W 5233	8 mos.	G 5720	*	*	*	9	5	6.5	42.4	259	285	4	4	4	0	0	Autopsied 19th day of gestation. Transferred to Standard Diet I at 3 mos.
		G 5632	*	*	*												
B 5264	8 mos.	GH 48	*	*	*	5	4	6.4	45.7	258	270						
		W 4609	*	*	*												

Totals: 54 rats.  
 85 positive matings.  
 80 litters of placental sign.  
 76 litters.

APPENDIX TABLE IV  
Reproductive Performance of Rats Reared on Standard Diet I (Whole Wheat, Casein, Whole Milk Powder, Sults and Milkfat)

Designation of Female	Age at Breeding	Designation of Male	Type of copulatory	Placental Sign (F. D. C.) Found	Litter	Number of Young		Average Wgt. of Young (in gms.)		Weight of Mother (in gms.)		Autopsy Record						Average Weight of Foetuses in Uterus	Notes
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora lutea		Living foetuses		Resorptions			
												R	L	R	L	R	L		
W 5418	5 mos	W 4872	•	•	•	5	5	6.4	47.8	210	262							6 more matings without placental sign. Autopsied. Infected.	
W 5423	6 mos.	5634 GH 00	•	•	•	8	5	5.6	38.0	225	249							Lung infection. Degraded.	
W 5424	6 mos	B 5649 BH 00 GH 79 B 51	•	•	•	7	6	6.3	47.8	286	300								
			•	•	•	10	6	6.0	52.0	324	329								
BH 5425	6 mos	5401	•	•	•	7	5	6.3	41.6	224	224							Autopsied 20th day of gestation.	
B 5429	5 mos.	BH 6554 W 1000	•	0	•	6	6	6.7	38.3	208	228							3 more matings without placental sign. Killed. Possibility of infection.	
B 5432	7 mos	G 1707 GH 4771 W 50	•	0	•	4	1	4.7	44.5	257	296							2 more matings without placental sign. Possibility of infection.	
W 5552	5 mos	W 5101 BH 4709 W 4872	•	•	•	6	4	6.0	52.5	252	235							Autopsied 19th day of gestation.	
			•	•	•	7	6	6.3	37.1	247	271							Autopsied 19th day of gestation.	
BH 5596	5 mos.	G 5716 W 5236 BH 79 BH 6554	•	0	•	4	1	7.0	59.2	268	285							Autopsied 19th day of gestation.	
W 5622	6 mos.	W 4640 BH 4709 BH 07 G 5230	•	•	•	6	0	6.8		254	251							Autopsied 19th day of gestation.	
			•	•	•	2	0	8.0	43.3	509	298								
			•	•	•	1	4	6.2											
BH 5623	6 mos.	W 4872 G 4707 BH 79 BH 4709 BH 69	•	•	•	9	6	4.9	41.1	241	250							Autopsied 19th day of gestation.	
			•	•	•	6	0	6.0		220									
			•	•	•	(4 dead) 6	5	6.2	41.2	262	219								



APPENDIX TABLE IV.—Continued

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Litter	NUMBER OF YOUNG		AVERAGE WGT. OF YOUNG (in gms.)			WEIGHT OF MOTHER (in gms.)		AUTOPSY RECORD						Average Weight of Foetuses in Grms.	NOTES
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora lutea		Living foetuses		Resorptions				
												R	L	R	L	R	L	R		
W 5628	7 mos.	G 67 B 5669	• •	• •	• •	5 (dead)	0	not weighed			181		6	6	6	0	0	2.4	Autopsied 19th day of gestation. Discarded.	
W 5635	5 mos.	W 5236 G 5716	• •	• •	• •	7 6	5 3	42.4 30.6	236 284	234 282								3 more matings without placental sign. Autopsied. Possibility of infection.		
W 5637	5 mos.	4872	• •	• •	• •	6 (1 dead)	4	5.0	31.7	204	222							1 more mating without placental sign. Autopsied. Possibility of infection. Discarded.		
W 5731	4 mos.	W 5101	• •	• •	• •	4	4	6.5	41.2	266	265							5 more matings without placental sign. Autopsied. Possibility of infection.		
BH 5727	4 mos.	W 4699	• •	• •	• •	7	6	5.6	42.6	200	248							5 more matings without placental sign. Autopsied. Possibility of infection.		
W 5734	7 mos.	W 4802 BH 4709	• •	• •	• •	2 7	0 6	5.0	34.0	300	325							5 more matings without placental sign. Autopsied. Infected.		
W 5736	4 mos.	W 4872 W 14 W 5236 GH 00	• •	• •	• •	7 (dead)	6	6.0	47.6	288 362	287							2 more matings without placental sign. Autopsied 11th day after mating. Possibility of infection.		
W 5738	4 mos.	BH 4709 W 1000	• •	• •	• •	9 8	5 6	5.1 5.7	43.8 41.0	236 280	263 280							2 more matings without placental sign. Autopsied. Infected.		
W 5744	5 mos.	W 5235	• •	• •	• •	?	0	not weighed		254								7 more matings without placental sign. Autopsied. Infected.		
W 5752	5 mos.	G 5230	• •	• •	• •	6	5	6.7	59.0	320	332							3 more matings without placental sign. Autopsied. Possibility of infection.		
G 5776	5 mos.	5236 W 5638	• •	• •	• •	5 (1 dead)	3	5.8	47.3	303	344							2 more matings without placental sign. Autopsied. Possibility of infection. Discarded.		
G 5890 W 5899 B 5905	50 days 7 mos. 50 days	G 5716 BH 6554 GH 6118 G 5718	• •	• •	• •	11 8 6	0 6 5	5.3 6 5.6	32.5 not weighed	191 236 170	220 201		?	5	3	1	0	4	3.0	Autopsied 19th day of gestation. Discarded.

APPENDIX TABLE IV—Continued

W 5928	6 mos.	GH 5865	•	0	•	6	5	6.0	49.0	281	320						Discarded. 2 more matings without placental sign. Autopsied. Infected.
B 5939	50 days	W 4802	•	•	•	3	?	not weighed	not weighed	288	?						
		W 4872	•	•	•	•	•	•	•	•	•						
W 5980	5 mos.	W 5101	•	•	•	3	3	7.7	46.0	329	354						Discarded.
BH 5908	5 mos.	B 5669	•	•	•	10	7	6.0	30.7	248	249						Discarded.
		W 5666	•	•	•	8	7	6.0	not weighed	268	?						Discarded.
G 6000	45 days	W 5717	•	•	•	9	6	6.0	not weighed	186	207						Discarded.
W 6026	7 mos.	W 02	•	•	•	4	3	6.0	39.3	252	253						
		G 45	•	•	•	•	•	•	•	•	•						
BH 6031	45 days	BH 5719	•	•	•	5	?	not weighed	not weighed	156	190						Autopsied 17th day of gestation. Discarded.
BH 6086	7 mos.	GH 77	•	•	•	?	?	weighed	weighed	288	300						
G 6101	5 mos.	BH 4769	•	•	•	2	0	5.0	•	294	•						Autopsied 18th day of gestation 1 more mating without placental sign. Autopsied. Possibility of infection.
BH 6126	40 days	G 5716	•	•	•	7	2	6.8	not weighed	151	not weighed						Discarded.
BH 6135	37 days	W 5717	•	•	•	9	?	5.5	42.0	170	217						Discarded.
BH 6145	7 mos.	BH 60	•	•	•	5	1	6.0	42.0	248	273						Discarded.
		BH 33	•	•	•	•	•	•	•	•	•						Autopsied 20th day of gestation.
W 6158	12 mos.	BH 27	•	•	•	5	5	6.0	45.0	274	309						
BH 6148	7 mos.	W 6251	•	•	•	?	4	not weighed	35.7	224	261						Autopsied 19th day of gestation.
		BH 79	•	•	•	•	•	•	•	•	•						Autopsied 20th day of gestation.
BH 6164	12 mos.	B 5669	•	•	•	•	•	•	•	•	•						
		W 46	•	•	•	•	•	•	•	•	•						
		W 46	•	•	•	•	•	•	•	•	•						
		BH 7307	•	•	•	2	2	7.0	51.0	272	283						
		BH 7305	•	•	•	•	•	•	•	•	•						
		W 26	•	•	•	•	•	•	•	•	•						
W 6166	12 mos.	BH 27	•	•	•	2	0	5.0	•	244	•						Autopsied 19th day of gestation.
		BH 66	•	•	•	(dead)	•	•	•	•	•						Autopsied 19th day of gestation.
W 6173	7 mos.	GH 77	•	•	•	•	•	•	•	•	•						Autopsied 19th day of gestation.
		W 22	•	•	•	•	•	•	•	•	•						Autopsied 21st day of gestation.
W 6206	7 mos.	W 5720	•	•	•	•	•	•	•	•	•						Autopsied 21st day of gestation.
		BH 50	•	•	•	•	•	•	•	•	•						
		W 02	•	•	•	•	•	•	•	•	•						
		GH 4771	•	•	•	7	6	6.0	36.1	208	240						Autopsied 19th day of gestation.
W 6214	7 mos.	BH 70	•	•	•	•	•	•	•	•	•						Autopsied 19th day of gestation.
BH 6230	7 mos.	GH 45	•	•	•	6	6	5.7	33.3	284	320						Autopsied 21st day of gestation.
		GH 00	•	•	•	•	•	•	•	•	•						Autopsied 21st day of gestation.
BH 6233	9 mos.	GH 00	•	•	•	6	6	6.0	35.5	255	250						Autopsied 19th day of gestation.
		BH 81	•	•	•	•	•	•	•	•	•						
		B 7307	•	•	•	2	0	4.0	•	304	•						
		W 29	•	•	•	•	•	•	•	•	•						
		G 00	•	•	•	1	0	4.0	•	332	•						

APPENDIX TABLE IV—Continued

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Litter	NUMBER OF YOUNG		AVERAGE WGT. OF YOUNG (in gms.)		WEIGHT OF MOTHER (in gms.)		Autopsy Record				Average Weight of Foetuses in Uterus	Notes
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora lutea	Living foetuses	Resorptions			
BH 6235	9 mos.	G 67 BH 79 W 00	•	•	•	8	4	5.2	35.2	257	255	5	2	0	0	2.3	Autopsied 19th day of gestation.
W 6239	9 mos.	BH 79 BH 25 BH 00	•	•	•	5	3	4.8	34.3	354	364	7	3	0	0	2.7	Autopsied 19th day of gestation.
W 6247	7 mos.	BH 00 W 00	•	•	•	4	4	6.0	39.7	384	385	8	5	0	0	2.4	Lung infection. Discarded.
W 6251	12 mos.	W 00 G 00	•	•	•	6	6	5.6	49.6	324	300	6	6	0	0	2.4	Autopsied 19th day of gestation.
W 6259	5.5 mos.	GH 77 BH 25 BH 00	•	•	•	6	6	6.7	28.1	256	223						Autopsied 2 days after mating. Normal.
B 6264	6 mos.	BH 4707 GH 60 BH 4770	•	•	•	d	5	6.5	43.8	272	256	5	4	3	1	3	3 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.
B 6269	7 mos.	W 27 G 5230 G 4707	•	•	•												Autopsied 19th day of gestation.
B 6285	9 mos.	BH 81 BH 5223 W 4694	•	•	•	6 (2 dead)	7	6.4	not weighed	374	373	1	5	4	3	3.2	Autopsied 20th day of gestation. 3 more matings without placental sign. Autopsied 2 days after mating. Infected.
W 6293	6 mos.	GH 77	•	•	•	6	0	4.5		210							4 more matings without placental sign. Discarded. Possibility of infection.
W 6536	10 mos.	W 7306	•	•	•	(dead)	3	6.7	60.3	331	352						Autopsied. Infected.
W 6605	10 mos.	G 30	•	•	•	8	6	6.5	31.3	310	306						4 more matings without placental sign. Autopsied. Infected.
BH 7397	4 mos.	W 94	•	•	•	8	5	5.0	43.0	280	280	6	6	2	1	3.0	Autopsied 19th day of gestation. 9 more matings without placental sign. Autopsied 2 days after mating. Infected.
W 7382	4 mos.	G 13	•	•	•	2 (1 dead)	0	6		276							
W 7445	2 mos.	G 68 B 7179	•	•	•	5 (2 dead)	2	4.0	27.0	156	178						

APPENDIX TABLE IV—Continued

		B 6145	.	.	0	3	3	6.7	53.3	234	250	4	3	1	0	0	0	4	5	4 more matings without placental sign—Autopsied 2 days after mating. Possibility of infection.
B 7450	5 mos.	W 93	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 20th day of gestation
W 7505	5 mos.	W 82	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation
B 7508	5 mos.	B 32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation
W 7590	4 mos.	W 6500	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation
B 7510	4 mos.	W 35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation
		W 83	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		W 89	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		BH 22	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		W 55	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
GH 7520	4 mos.	B 50	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation, 5 more matings without placental sign. Autopsied. Possibility of infection.
		W 99	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
GH 7530	4 mos.	W 45	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation.
W 7560	4 mos.	G 09	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation. Lung infection. Autopsied 2 days after next mating. Normal.
W 7561	4 mos.	B 7175	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		G 09	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
BH 7665	5 mos.	B 6706	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 20th day of gestation, 4 more matings without placental sign. Autopsied. Infected.
W 7877	4 mos.	W 83	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		W 7306	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
G 7880	4 mos.	BH 00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation. Infection on right side.
		B 7175	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
G 7881	4 mos.	W 35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		W 00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
W 7882	3 mos.	W 5100	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 13th day of gestation
		G 10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 14th day of gestation.
W 7895	5 mos.	W 91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		B 6706	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		W 5125	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 10th day of gestation
G 7928	3 mos.	W 94	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		W 02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 19th day of gestation.
G 7929	3 mos.	W 6500	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
		W 82	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 15th day of gestation.
		W 5400	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
B 7930	3 mos.	W 30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Autopsied 20th day of gestation.

Totals: 77 rats.  
166 positive matings.  
138 findings of placental sign.  
135 litters.



# THE PRODUCTION OF STERILITY WITH NUTRITIONAL REGIMES ADEQUATE FOR GROWTH AND ITS CURE WITH OTHER FOODSTUFFS.\*

By  
HERBERT McLEAN EVANS  
and  
KATHARINE SCOTT BISHOP.

*From the University of California and Dairy Division, Bureau of Animal Industry, United States Department of Agriculture.\*\**

- A. Introduction.
- B. The Production and Analysis of Sterility on the Basic Ration.
  - 1. *Competency of the well known basic ration to furnish normal growth in the rat.*
  - 2. *Incidence of sexual maturity and the ovulation rhythm of animals reared on the basic ration.*
  - 3. *Fecundity and fertility on the basic ration.*
- C. The Cure of Proven Dietary Sterility by the Addition to the Basic Ration of Certain Natural Foodstuffs.
- D. The Prevention of Dietary Sterility by Rearing Animals on the Basic Ration to Which is Added a Single Natural Foodstuff.
- E. The Dietary Cause of Sterility on the Basic Ration.
  - 1. *Possible deficiency of the basic mixture in known substances.*
  - 2. *Components of curative foods, other than vitamins, which may be responsible for the cure.*
  - 3. *Segregation of the fertility conferring factor X from the vitamins hitherto known.*
- F. Summary.

## *A. Introduction.*

Inability to bear young, the condition broadly and simply designated as *sterility or infertility*, may be due to disturbance of any one of several members of the chain of events involved in mammalian reproduction. Disturbances in insemination, in ovulation, in germ cell vigor, implantation, normal placental function or in parturition have each to be considered before we are at liberty to assign sterility to instances of breakdown in any one of the links in this rather complex chain. Any scientific study of fertility must attempt to segregate these factors in the physiology of the reproductive process, for upon our ability to

\* On the relations between fertility and nutrition IV.

\*\* Work aided by grants from the Committee for Research on Sex Problems of the National Research Council and the California State Dairy Council. The writers wish to express their especial thanks to Mr. C. E. Gray of San Francisco and to Dr. C. W. Larson and Mr. L. A. Rogers of Washington.



make such a separation may depend the recognition of separate physiological relationships or needs, for instance, of the ovarian and of the uterine mechanism. In the previous papers of this series we have already hinted at our discovery that the ease of breakdown in these two mechanisms is not identical. In this paper we hope to demonstrate that the classic nutritional regime with so called "purified" foodstuffs and the two well known vitamins, A and B, while permitting normal growth of the rat, produces sterility and that it produces this without upset to fecundity (since ovulation occurs and a normal number of ova are fertilized and implanted), but by the production of a highly peculiar deficiency disease which always affects the placenta and either secondarily or, even, primarily the products of conception. We are able further to show that the disease is rapidly cured by the administration of certain natural foodstuffs.

*B. The Production and Analysis of Sterility on the Basic Ration.*

It is well recognized that the detection of vitamins, more particularly the substances now designated fat soluble A and water soluble B, was greatly facilitated if not actually conditioned by the employment of food mixtures possessing the three fundamental foodstuffs (fats, carbohydrates and proteins) in separate, relatively pure form. Nutritional studies with rats have thus long been conducted with food mixtures composed of casein, lard and cornstarch. If such a basic diet is employed with an adequate salt admixture and appropriate daily rations of vitamins A and B in the form of butter and yeast, these animals maintain sleek coats and every other appearance of perfect health and activity and exhibit a growth which compares favorably with that described by most observers as normal for the species. The diet, for instance, which we have employed is

Basic Ration\*

(Standard Diet II)

casein . . . . .	18
cornstarch . . . . .	54
lard . . . . .	15
milkfat . . . . .	9
salts . . . . .	4
.4 — .6 gram dried yeast daily	

\* The exact source of our various ingredients is as follows.

Casein supplied by the California Central Creameries and is a high grade commercial lactic acid product. It is dried at a temperature not above 55° C. It is important to note

that all casein has been produced from milk coming from cows fed on green pasture, and contains a very small percentage of milkfat. As would be expected, we have found it to contain some vitamin A as well as vitamin B. Throughout these experiments, except where noted, we have used the commercial product without extracting or submitting it to oxidation or high temperature (other than that involved in cooking and drying).

*Cornstarch* put up in one pound lots by the National Starch Company, successor to T. Kingsford and Son, Oswego, New York.

*Milkfat* made by the California Central Creameries, with an exceedingly low content of protein and water, said to be 99.97 per cent. pure. The milkfat was separated at the plant by centrifugal force. In all cases, fresh whole milk was delivered twice daily and was invariably less than four hours old so that a relatively low bacterial count was present. In no case had acidity been permitted to develop.

*Lard*, ordinary commercial products made by Armour and Company, labeled Shield Pure Lard, and by Swift, labeled Swift's "Silverleaf" Brand Pure Lard, were used.

*Salts*. The salt mixture employed was identical with that used by E. V. McCollum and consisted of

NaCl	. . . . .	0.173
MgSO <sub>4</sub> (anhyd)	. . . . .	0.266
NaH <sub>2</sub> PO <sub>4</sub> +H <sub>2</sub> O	. . . . .	0.347
K <sub>2</sub> HPO <sub>4</sub>	. . . . .	0.954
CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> +H <sub>2</sub> O	. . . . .	0.540
Fe citrate	. . . . .	0.118
Ca lactate	. . . . .	1.300

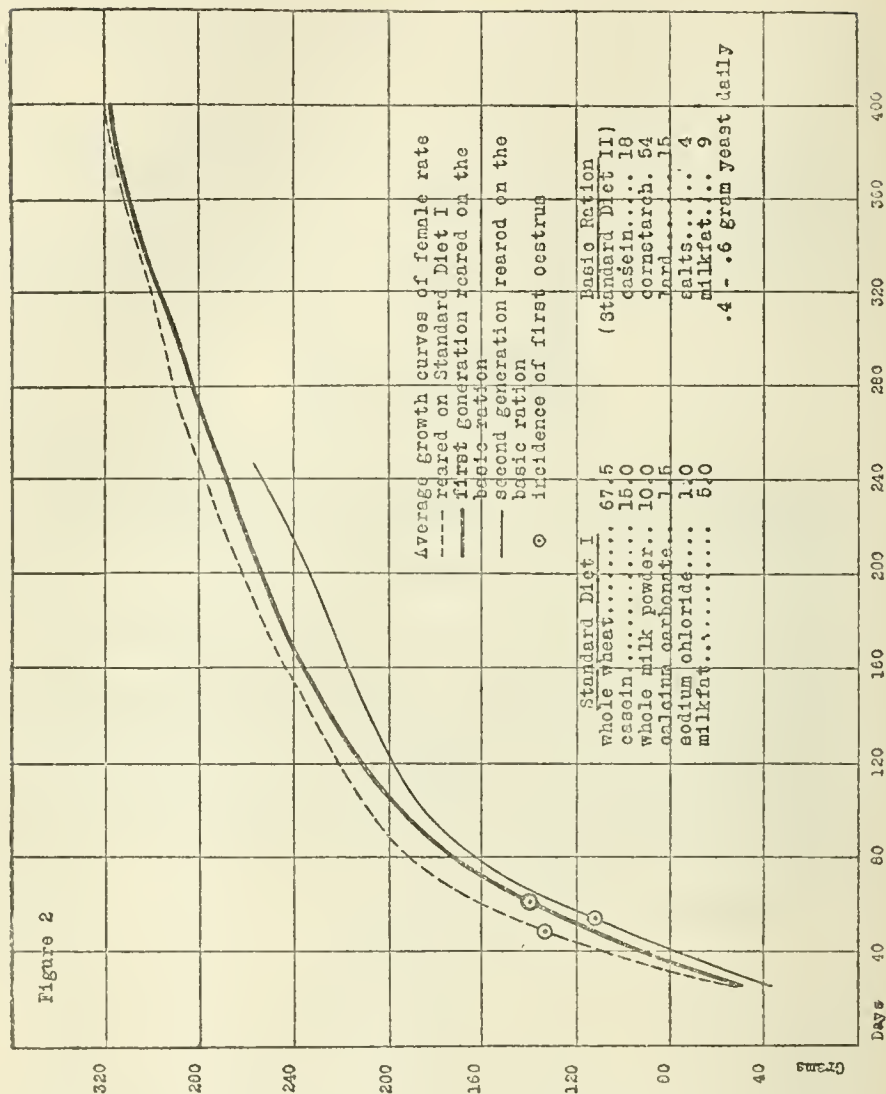
The salts purchased by us bear the following labels: Sodium Chloride—Highest purity "C. P." Merck; Magnesium Sulphate—Highest purity—dried, Merck; Sodium Phosphate Monobasic (biphosphate) Merck; Potassium Phosphate Dibasic—Highest purity "C. P." Merck; Calcium Phosphate Monobasic (biphosphate) Merck and Mallinckrodt; Ferric Citrate—Iron Citrate U. S. P. VIII—scales, Merck and Mallinckrodt; Calcium Lactate, U. S. P. IX, Merck.

Yeast was secured from the Harris Laboratories and is called by them, "Tested dried yeast. Tested for vitamin B activity." It was always fed separately, daily, in a clean glass dish within a clean metal box.

To serve as a complete record we also submit the following detail of our preparation of the food—120 grams of the salt mixture (see above) is added to approximately five quarts of water and brought to boiling on a gas stove. In the meantime 1620 grams of cornstarch and 540 grams of casein are dissolved in six quarts of water (never hot enough to gelatinize the cornstarch). Gelatinizing is accomplished by adding the cornstarch-casein solution to the salt solution when the latter is in active ebullition, the mixture being stirred vigorously for from three to five minutes, in which time the gelatinization is complete; the temperature of the mixture reaches 90° C. It is removed from the stove and spread in shallow enamel pans in sheets about one and a half inch in thickness, and the pans then placed on steam pipes which are enclosed in a metal case so as to constitute a drying oven; the air current is sluggish so that three days are consumed in drying. The actual temperature of the steam pipes never rises above 120° C. The dry food is fed into the granulator side of a Hobart mill and the ground material finally mixed with 450 grams of lard and 270 grams of milk fat.

# 1. Competency of the well known basic ration to furnish normal growth in the rat.

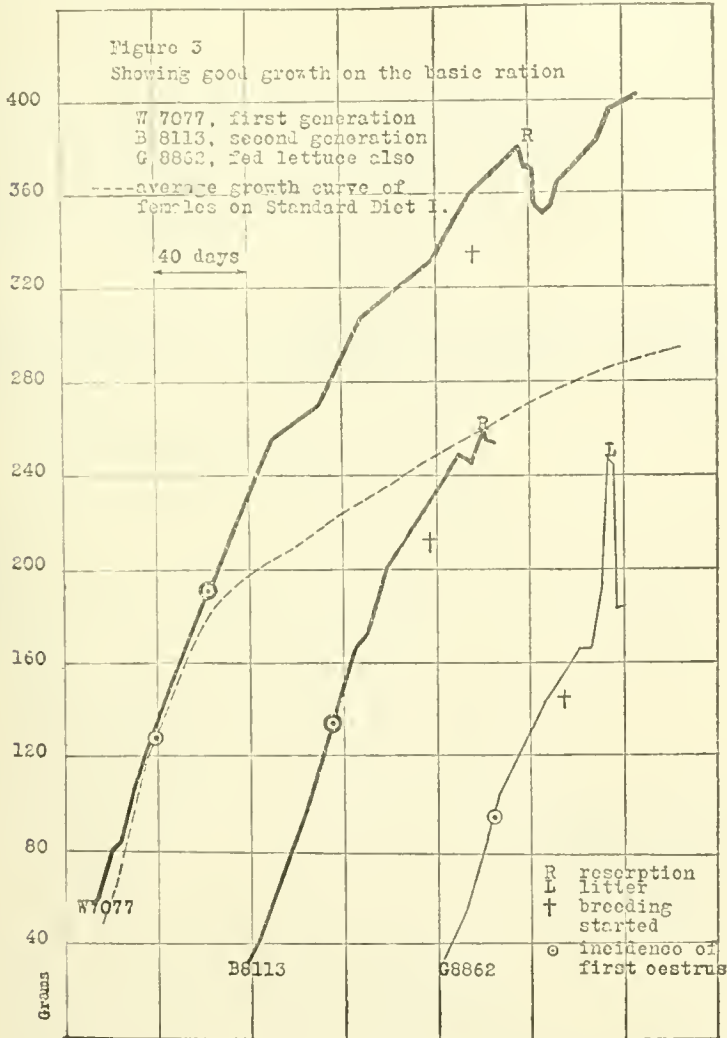
We present in Figure 2 the curve of average growth of the female rats held upon this ration the study of whose fertility is the special object of these experiments, together with a similar curve of average growth obtained with the whole wheat-whole milk-casein mixture, the standard diet found so excellent for growth and reproduction (McCollum) and which we have designated Standard Diet I. The apparent slight inferiority in average growth on the basic ration which becomes more evident in the second generation, is without significance when we view the considerable variations in growth obtained upon the excellent Standard Diet I itself. Upon the latter regime a large number of



entirely normal animals grow at the rate depicted in the lowest curve. The fact that the curves of Figure 2 represent average values obtained from groups too small to furnish statistical results always becomes apparent when we study the growth curves of single individuals. Furthermore, in these groups we had not the advantage of comparing the behavior of littermate sisters. A rather extensive experience has convinced us that such

animals tend to grow at a more nearly uniform rate than do animals chosen at random even within the same colony.\*

A study of the growth performance of individual animals on the basic ration (Standard Diet II) shows clearly that the rapid



growers here may greatly exceed the average upon the standard ration (Standard Diet I) and in Figure 3 we have depicted with dark lines the growth curves of two such females on the basic

\* In all the special modifications of our basic ration (Standard Diet II) which we have employed, we have invariably used littermates as controls.

cornstarch-casein-lard mixture, belonging to the first and second generation respectively. The dotted line shows average performance upon Standard Diet I. We may remark at once that both of these animals suffered from the sterility deficiency disease which we have to report for this and similar basic diets. The last curve, in lighter line, is that of an individual reared on the same ration but fed in addition a small amount of a single natural foodstuff, in this case lettuce, which served as a prophylactic against the disorder, so that normal fertility resulted. It will be seen that fertility effects were secured without acceleration of the growth impulse, and in our experience the two phenomena, growth and

TABLE I

Showing the Time of Incidence of First Oestrus In Animals Reared On the Basic Ration (Standard Diet II) and In Those Reared On a More "Natural" Ration (Standard Diet I). (The Detailed Histories Are given In Appendix Tables I and II)

Diet	Number of Instances of First Oestrus Occurring In the Indicated Time Intervals										Total Number of Rats
	Days:										
	32 to 36		37 to 55		56 to 66		67 to 79		82 to 175		
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	
<b>BASIC RATION (Standard Diet II)</b> (Casein 18, Cornstarch 54, Lard 15, Milkfat 9, Salts 4, Yeast .4-6 Gram Daily)											
<i>First Generation</i> . . . . .	31	6%	109	56.8%	34	17.7%	25	13.0%	21	10.9%	192
<i>Second Generation</i> . . . . .	24	5%	27	61.4%	10	22.7%	4	9.1%	1	2.3%	44
<b>STANDARD DIET I</b> (Wholewheat 67.5, Casein 15, Whole Milk Powder 10, Sodium Chloride 1, Calcium Carbonate 1.5, Milkfat 5)											
	18	3.1%	459	80.5%	73	12.8%	20	3.5%	0	0.0%	570



reproduction, are not indistinguishably related. *We may summarize our data on the growth of these three groups of animals by stating that the performance on the so-called basic or "purified" ration falls well within the limits of growth upon the best possible regime.\**

2. *Incidence of sexual maturity and the ovulation rhythm of animals reared on the basic ration.*

During the time we were administering daily about .3 gram only of dried whole yeast we reported the retardation of gonadal activity in animals held upon the basic ration (J. Met. Res., March, 1922).† The first oestrus in 570 animals on Standard Diet I was in 84 per cent. of the individuals at some time between the thirty-second and fifty-fifth day of life. The majority of individuals held on the basic diet also mature in this time interval, but, as Table I shows, only about 60 per cent. did so, the remainder maturing later, and many of them later than is ever experienced for Standard Diet I.‡

The retardation of gonadal activity is always shown more clearly by a study of the oestrous rhythm. This has been impaired in many of our animals held on the basic ration. The animals reported by us nine months ago (J. Met. Res., March, 1922) had only about 55 per cent. of their oestrus cycles of five days or less in length, over against 75 per cent. of such cycles in animals maintained on Standard Diet I. When this group was

\* We disagree with the recent statements of Kennedy and Palmer who hold that yeast is an unreliable and usually inadequate source of water soluble vitamin B. Before the present study was inaugurated we had experienced evidence of the unsatisfactory performance of animals on the basic ration when dried whole yeast was administered in 200 milligram doses from single yeast lots which we employed for over a month. The employment of fresh Harris yeast, secured in fortnightly shipments and fed in from .5 to .6 gram daily doses has yielded the growth which we have just shown reasons for believing to be perfectly normal. We may add that in a special lot of animals where littermate sisters were fed identically save for differences in their daily yeast doses (.4 gram and 1 gram) there was no consistent growth stimulus which could be ascribed to the higher dose, some instances, in fact, occurring where slightly superior growth was shown on the .4 gram dose.

† In the table on page 341, Volume I of this Journal, the figure 99 for the per cent. of 4 and 5 day cycles in rats on Standard Diet 11 (the basic ration) should read 66 instead 99. It will be noted from this table (when corrected) that only from 42 to 68 per cent. of oestrous cycles in animals reared on the basic ration were of 4 or 5 day lengths whereas on Standard Diet I, about 75 per cent. of cycles are of this length.

‡ However, from a rather extensive experience we would wish to emphasize the fact that the time of first oestrus is subject to very considerable fluctuation, even in the case of littermate sisters maintained on the same ration, and that only a considerable departure from the mean time can be regarded as significant. A profound variation is always of significance. We would, for instance, refer here to the very late maturity (in spite of good growth) which is exhibited by animals reared solely on milk. In this group, late maturity heralds an interference with physiology which would be obscured by the excellent growth, an interference further emphasized by irregularity in the oestrous rhythm. Furthermore, we can state that many clearly defective dietary regimes do not significantly postpone the incidence of the first oestrus. This is, for instance, the case with several regimes employed by us where fat soluble vitamin A is low.



combined with a somewhat later and more favorably handled group, the figure secured was 61.2 per cent. of such cycles, as seen in Table II (first generation, 141 rats).

TABLE II

Showing Length In Days of Oestrous Cycles. (The Detailed Histories Are Given In Appendix Tables I and II)

Diet	Cycles 3-5 Days In Length		Cycles 6 Days In Length		Cycles 7 or More Days In Length		Total Number of Cycles
	Number	Percentage	Number	Percentage	Number	Percentage	
<b>BASIC RATION (Standard Diet II)</b>							
(Casein 18, Cornstarch 54, Lard 15, Milkfat 9, Salts 4, Yeast 4-6 Gram Daily)							
<i>First Generation</i> (141 Rats).....	1163	61.2%	340	17.9%	398	20.9%	1901
<i>Second Generation</i> (40 Rats).....	163	68.8%	47	19.8%	29	11.4%	237
<b>(Standard Diet I)</b>							
(Whole Wheat 67.5) Casein 15, Whole Milk Powder 10, Sodium Chloride 1, Calcium Carbonate 1.5, Milkfat 5) (570 Rats) .....	7509	75.1%	1439	14.4%	1052	10.5%	10,000

The preceding section will, we hope, have demonstrated that these animals grow at a normal rate and that we are consequently unable to confirm the statement that dried yeast does not give adequate growth and is hence defective as a source for water soluble vitamine B. When Kennedy and Palmer report clear interference with growth on the samples of yeast tested by them, we must accept their conclusion that their material was defective as a proper source for B. But these authors seek to further support the contention that yeast has insufficient B by adducing the failure of their rats to reproduce normally. We hope to show in the present study that defective reproduction on the basic ration

is not due primarily to inadequacy of B but to the absence of a specific factor needed for placental function, which we have provisionally designated by the letter X. Hence neither of the reasons advanced against the use of yeast as a source of vitamin B would appear to be adequate. We have secured reproduction without increase of B other than that present in 0.4 gram fresh dried yeast daily by the administration of the factor X as contained in a very high proportion of milkfat (24 per cent.).

Our discovery of the interference with gonadal activity observed in all our earlier work on the basic ration cannot be referred with certainty to insufficient B. These animals grew normally. *The study of ovulation rhythm furnishes a new, a different and a more sensitive test of physiological well-being than that furnished by growth rate. With us, animals that grow normally, or at least within the limits to be recognized as those of the normal rate, have repeatedly been so seriously impaired in gonadal activity as to depart widely from the normal ovulation rate.*

More yeast improves the ovulation rate. In our more recent work with increased yeast dosage (approximately 0.6 gram daily) and with fortnightly renewed samples of freshly dried yeast the proportion of short oestrous cycles shown by animals on the basic ration has approached 70 per cent. (Table II, second generation rats). We believe but are obviously not yet prepared to state that an extensive research with sufficient yeast dosage in Standard Diet II and with littermate sister controls on Standard Diet I, would show an identity in gonadal physiology in the two groups. Yet those on the former diet would be sterile. Very recently (i. e., within the last three months) we have studied the time of first oestrus in forty-five sterile animals maintained on the basic ration and as many as 86 per cent. of them occurred within the time interval regarded by us as normal from our previous study of 570 normal animals reared on Standard Diet I (J. Met. Res., Feb. 1922). The oestrous cycles in the forty-five animals were not studied since they were used for breeding experiments, but it is our conviction that they would also have exhibited a close approach to normality in frequency. Thus, since yeast does not contain the substance need for placental function, we may safely feed larger amounts of it without disturbing the sterility disease, and such larger amounts may give normal gonadal function. We are not prepared to state whether such an improvement in ovula-

tion rhythm is actually due to increased B. We are engaged in testing this point but may point out that possibly something other than B may be necessary for complete ovarian normality.

It may be said, in summary, that we have shown that unless generous quantities of yeast are fed with the basic ration, normal gonadal rhythm may not result.\* Yet this slowing of ovulation is unrelated to the reproductive failure resulting from such a ration. *When the ovulation rate is normal, the reproductive failure still persists.* As we shall show, a deficiency which manifests itself after fertilization is the explanation of the sterility observed in these animals and this cannot be due to insufficient B, for no amount of yeast (e. g. 25 per cent.) cures the disorder and probably all of our curative substances contained less vitamine B than such a great yeast dosage; this is, for instance, certainly the case with 24 per cent. pure milkfat. It is, furthermore, pertinent to our analysis of the biological fault in the dietary sterility produced by the basic ration, that the particular animals (those of the second generation) showing normal or approximately normal gonadal rhythm were the ones exhibiting absolute sterility. It is hence evident that improvement in ovarian behavior went hand in hand with deterioration of the uterine mechanism and the development of complete failure to produce young.

### 3. *Fecundity and fertility on the basic ration.*

Animals held on the basic ration are usually sterile. In the earlier portion of our work we secured a number of instances of partial fertility so that some (44) second generation females were available for study. These animals are invariably sterile, and recently all of the first generation females held upon the basic ration are similarly entirely sterile. It will be important for us to demonstrate to what degree this sterility is due to ovarian and to what degree to uterine malfunction. The reader will have already seen that there is frequently some departure from normal sexual maturity and normal ovulation rhythm in these animals. *But that the early steps in reproduction are not gravely interfered with, or at any rate are not obliterated, is shown by the occurrence of the "placental sign"† on the four-*

\* Even without increased yeast dosage, we had repeatedly secured exceptional individuals on the basic ration showing early maturity and a long succession of perfect four and five day oestrous cycles.

† A slight leakage of blood from the placenta found in all cases of gestation and occurring normally from the 14th to 16th day after insemination. Recognized with the vaginal speculum or by the presence of erythrocytes in the smear. See the Normal Reproductive Performance of the Rat. Jr. Met. Research, Vol. III, No. 2, Feb. 1923

teenth day after a positive mating in as large a proportion of the sterile animals as of completely fertile rats (see implantation per cent. figures in Table III). Autopsies performed at this time show that the sign has, as usual, been given by placentae, although

TABLE III\*

Showing Disappearance of Fertility In Rats Reared On the Basic Ration First and Second Generations, In Comparison With the Fertility of Rats Reared On Standard Diet 1 (Whole Wheat-Whole Milk Powder-Casein-Salts-Milkfat)

Diet	Number of Rats	Number of Positive Matings	Number of Findings of Placental Sign	Number of Litters	Group Fertility per Cent	Implantation per Cent	Placental Index
<b>STANDARD DIET I</b> (Whole Wheat 67.5, Casein 15, Whole Milk Powder 10, Sodium Chloride 1, Calcium Carbonate 1.5, Milkfat 5) . . .	77	166	138	135	81%	83%	98%
<b>BASIC RATION</b> (Standard Diet II) Casein 18, Cornstarch 54, Lard 15, Milkfat 9, Salts 4, Yeast .4-.6 Gram Daily)							
<i>First Generation**</i> Total . . . . .	108						
First Period Sept., 1921- May, 1922 . . . . .		121	101	60	50%	84%	59%
Second Period May, 1922- Dec. 20, 1922 . . . . .		90	76	2	2%	84%	3%
<i>Second Generation</i> May, 1922- Sept., 1922 . . . . .	34	63	57	0	0%	90%	0%

\* See page 14a.

\*\*It is our feeling that this apparently inexplicable difference in fertility on the basic ration during various experimental periods is possibly seasonal. It is not a genetic variation, since rats from many litters behave similarly, nor yet due to individual variations in requirements for reproduction since many individuals which were partially fertile in the earlier period, in the latter period were completely sterile. We must attribute it to variations of content in the unknown factor, X, present in the foodstuffs used, possibly in the milk products, casein, or milkfat, as it will be developed in the following pages that whole milk contains a low amount of the essential factor, X, particularly in its fat. At the present time we are again securing occasional litters on the basic ration, with, however, young of such low vitality as not to survive more than two or three days even when suckled. Further work, and work with extracted foodstuffs, will be necessary to discover the reason for such sporadic fertility on the basic ration.

TABLE IV

Showing the Numerical Relations Between Ova Shed (Corpora Lutea) and Those Implanted Either Successfully (Living Foetuses) or Unsuccessfully (Resorptions) In Rats On a Good Nutritional Regime and In Those On the Basic Ration

Diet	Number of Cases	Number of Corpora	Number of Living Foetuses	Number of Resorptions	Average Number of Corpora per Case	Average Number of Foetuses per Case	Average Number of Resorptions per Case	Percentage of Ova Implanted	Percentage of Implantations Resorbed
<b>STANDARD DIET I</b> (Whole Wheat 67.5, Casein 15, Whole Milk Powder 10, Sodium Chloride 1, Calcium Carbonate 1.5, Milkfat 5) . . . . .	36	365	226	27	10.1	6.3	.75	68%	10.7%
			253			7.05			
<b>BASIC RATION</b> (Standard Diet II Casein 18, Cornstarch 54, Lard 15, Milkfat 9, Salts 4, Yeast .4-6 Gram Daily). . . . .			64	268		1.3	5.4		
<i>First Generation</i> . . . . .	50	487	332		9.7	6.7		68%	81%

these are very abnormal and there may have even been complete resorption of the foetuses. The extent to which in these cases the normal number of eggs is matured per ovulation can only be determined by autopsies and counts of the corpora lutea. Fifty such cases have been autopsied at a sufficiently late time in gestation for us to be certain of the corpora lutea of pregnancy. Table IV gives the counts which have been made and the number of implantations, normal and otherwise. When these data are compared with the data secured from almost the same number of cases maintained on Standard Diet I, grounds are found for our conviction that in our sterility disease there is no interference with the average number of eggs liberated, with their fertilization or, indeed, with the occurrence of implantation.

### C. The Cure of Proven Dietary Sterility by the Addition to the Basic Ration of Certain Natural Foodstuffs.\*

#### a) Cures with a green leaf—lettuce. Natural foodstuffs con-

\* The rats reported upon in this section are first and second generation animals reared on the basic ration. Their age at first oestrus and ovulation records are given in Appendix Tables I and II; their reproductive performance on the basic diet in Appendix Tables III and IV and on the modified diets in Appendix Table V.



tain a factor, X, which prevents the development of the sterility produced by the basic regime or which cures the disorder. We were first acquainted with this in our desire to ascertain whether the antiscorbutic vitamine C was to any degree responsible for our results. To some animals we administered orange juice and to others fresh green leaves of lettuce and were surprised to find a comparatively sudden restoration of fertility in the latter group. This phenomenon was then made the object of special study. Animals reared and maintained on the basic regime were tested in order to prove the presence of sterility in each individual. After one or more resorptions, there was introduced into the cage in each instance, daily, 40 grams of the fresh green leaf of lettuce, an amount which would give a dried weight of about 1.4 gram. In all cases uncomplicated by infection this single added dietary component evoked a restoration of fertility, as shown in the seven cases detailed in Table V.

TABLE V

Showing the Reproductive Performance of Rats Reared On the Basic Ration and, After Sterility Was Proven, Fed In Addition 40 Grams of Fresh Lettuce Leaves Daily

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations on the Modified Basic Diet
BH 8142	2 Resorptions	3 days before mating	Litter of 5 (1 dead), 4 weaned Litter of 8, 5 weaned*
W 7871	1 Resorption	1 day before mating	Resorption Litter of 7, 6 weaned Litter of 12 (1 dead), 6 weaned
W 8152	2 Resorptions	2 days after mating	Litter of 4 (1 dead), 2 weaned Litter of 5, 5 weaned
W 8327	3 Resorptions	4 days before mating	Litter of 1 (dead) Litter of 5 (1 dead), 4 weaned
W 7873	1 Resorption	1 day before mating	Resorption Litter of 8, 6 weaned
W 8239	1 Resorption	4 days before mating	Litter of 3 (1 dead), 2 weaned Litter of 2, 2 weaned
W 8174	1 Resorption	4 days before mating	Litter of 8, 6 weaned

Summary: 7 rats used in experiment

\* Where the young were in excess of six only six were given to be suckled, so that when this number was weaned the lactation performance is above average.



Summary: 7 rats used in experiment

On the basic ration:

11 resorptions

0 litters

On the modified basic ration:

2 resorptions

12 litters

Total number of young born.... 68

Number of young born dead.... 6

Number of young weaned..... 48

\*Average weight at birth. 5.8 gms.

Average weight at weaning ..... 34.6 gms.

Percentage gain in mother's weight during lactation ..... 3.4%

It will be noted that in most cases lettuce feeding was instituted only a few days before the trial gestation was inaugurated. Two of the best cases occurred where the curative foodstuff was added to the ration but one day before mating and it is of interest that in one case (W 8152) the treatment was begun two days after mating. These cases and, especially the last one, would appear to furnish conclusive evidence that the placental disease is not occasioned by defects in the products of conception but that the latter are to be regarded as healthy. It is otherwise difficult to explain the successful outcome in the last case, for the curative

TABLE VI

Showing the Reproduction Performance of Rats Reared On the Basic Ration and, After Sterility Was Proven, Fed In Addition 10 Grams of Raw Lean Beef Muscle Daily

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
BH 8120	1 Resorption	6 days before mating	Resorption Litter of 3, 1 weaned
B 7737	1 Resorption	1 day before mating	Resorption? (Lost 17 gms.) Resorption Resorption? (Lost 14 gms.)
W 8172	2 Resorptions	6 days before mating	Litter of 3 (1 dead) 0 weaned Litter of 3, 1 weaned
GH 8252	1 Resorption	Day of mating	Litter of 10, 6 weaned Litter of 11, 5 weaned

\* The reader will find "normal" values in our paper III of the present series "The Normal Reproductive Performance of the Rat." J. Met. Res., Vol. III, No. 2, p. 201.

Summary: 4 rats used in experiment

On the basic ration:

5 resorptions

0 litters

On the modified basic ration:

4 resorptions (2 questionable)

5 litters

Total number of young born... 30

Number of young born dead.... 1

Number of young weaned..... 13

Average weight of young

at birth ..... 5.7 gms.

Average weight of young

at weaning ..... 36.8 gms.

Percentage gain in mother's weight during lactation .....

9.9%

food could hardly have changed diseased to healthy ova after ovulation had occurred.

b) *Cures with fresh meat.* Fresh meat, represented by the relatively fat-free musculature of the jaw of the cow was fed in daily 10 gram quantities and also in excess as a single foodstuff added to the basic ration. Four cases are detailed in Table VI. It will be noted that in three of them fertility has resulted, in one case promptly, a large litter (10) resulting when the administration of meat was not begun until the day of insemination. Work which we will report in a later section of the paper and in which animals were reared with a "prophylactic" daily dose of meat resulted in such unequivocal success that we are disposed to regard this substance as possessed of the same element which confers fertility in the case of lettuce, even though quantitative comparisons are necessary for determining its relative abundance.

TABLE VII

Showing the Reproductive Performance of Rats Reared On the Basic Ration and, After Sterility Was Proven, Transferred to This Diet Modified so as to Contain 50% Whole Wheat

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
W 8438	1 Resorption	4 days before mating	Litter of 6, 5 weaned
GH 8432	1 Resorption	5 days before mating	Litter of 3 (1 dead), 2 weaned
G 8331	1 Resorption	3 days before mating	Litter of 3, 3 weaned

Summary: 3 rats used in experiment

On the basic ration:

3 resorptions

0 litters

On the modified basic ration:

0 resorptions

3 litters

Total number of young born... 12

Number of young born dead.... 1

Number of young weaned..... 10

Average weight at birth. 5.7 gms.

Average weight at weaning ..... 41.6 gms.

Percentage gain in mother's weight during lactation .....

3.6%

c) *Cures with whole wheat.* In order to demonstrate whether or not cereals contain a beneficial substance, we added to the basic ration one-half by weight of whole ground wheat. The three cases treated in this way are given in the subjoined Table VII and again show the return of fertility due to the added food element.

d) *Cures with wheat embryo.* A surmise that the beneficial factor in wheat might lie, as is the case with the water soluble vitamine B, in the germ or embryo, led us to add one-third by weight of wheat embryo\* to the basic ration. An animal of proven sterility when given the new diet, beginning only a single day before mating, showed restoration of fertility.

TABLE VIII

Showing Performance of a Rat Reared On the Basic Ration and, After Sterility Was Proven, Transferred to This Diet Modified So as to Contain  $\frac{1}{3}$  Wheat Embryo by Weight

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
W 8719	1 Resorption	1 day before mating	Litter of 7, 6 weaned

Average weight of young at birth ..... 5.9 gms.

Average weight of young at weaning ..... 41.4 gms.

Percentage gain in mother's weight during lactation ..... 3.3%

\* We are indebted to Dr. C. B. Kress and to the Sperry Flour Company of Vallejo, California, for frequent deliveries of fresh lots of wheat embryo used in our work.

e) *Failure to cure with milk.* The conspicuous excellence of milk as far as vitamins A and B are concerned led us to attempt to treat disabled animals with this product. The cures were attempted with fresh pasteurized milk, which was placed in the

TABLE IX

Showing the Reproductive Performance of Rats Reared On the Basic Diet, and, After Sterility Was Proven, Fed In Addition An Excess of Fresh Pasteurized Milk Daily

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
W 7724	1 Resorption	16 days before mating	Resorption
BH 7727	1 Resorption	8 days before mating	Resorption Resorption Litter of 1 (dead) Resorption
W 7962	2 Resorptions	2 days before mating	Resorption

Summary: 3 rats used in experiment

On the basic ration

4 resorptions

0 litters

On the modified basic ration:

5 resorptions

1 litter

Total number of young..1 born dead

Weight of young.....3 grms.

TABLE X

Showing the Reproductive Performance of Rats Reared On the Basic Diet, and, After Sterility Was Proven, Transferred to This Diet Modified so as to Contain 25% Whole Milk Powder (Merrill-Soule, Syracuse, N. Y.)

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
B 7959	2 Resorptions	5 days after mating	Resorption Resorption Litter of 5, 5 weaned*
W 7862	1 Resorption	4 days before mating	Resorption Resorption
GH 7889	1 Resorption	1 day after mating	Resorption Litter of 3, 0 weaned Resorption

\* Three females of this litter were successfully reared on the same diet, but proved sterile, all resorbing their first litters. See Appendix Table V, 28999, G9000, G9001

## Summary: 3 rats used in experiment

On the basic ration  
4 resorptions  
0 litters

## On the modified basic ration:

6 resorptions  
2 litters  
Total number of young born..... 8  
Total number of young weaned.. 5  
Average weight at birth.. 5.5 gms.  
Average weight at wean-  
ing ..... 38.2 gms.  
Percentage gain in moth-  
er's weight during lac-  
tation ..... 26.9%

cage to be consumed ad libitum, and also by adding to the basic ration one-fourth or one-third by weight of Merrill-Soule whole milk powder. In all, ten individuals have been tested and, to our surprise, the failure of milk has been pronounced.

TABLE XI

Showing the Reproductive Performance of Rats Reared on the Basic Ration and, After Sterility Was Proven, Transferred to This Diet Modified so as to Contain  $\frac{1}{5}$  Whole Milk Powder (Merrill-Soule, Syracuse, N. Y.)

Designa- tion of Rat	Gestations On the Basic Ration	Time of Modifica- tion of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
G 7734	1 Resorption	4 days before mating	Resorption
G 8271	3 Resorptions	6 days after mating	Resorption Resorption Resorption
G 8279	2 Resorptions	7 days after mating	Resorption Resorption
W 8280	3 Resorptions	11 days after mating	Resorption Resorption Resorption

## Summary: 4 rats used in experiment

On the basic ration  
9 resorptions  
0 litters

## On the modified basic ration:

9 resorptions  
0 litters\*

f) *Failure to cure with orange juice.* Four sterile animals were given daily 16 cc. of the freshly expressed unfiltered juice of oranges. Inasmuch as Davey (1921) estimates that 1.5 cc. of

\* June 2, 1923; since these experiments were completed, others have been performed by us with milk from cows upon the green feed so abundant locally from February to May. These studies have now shown a low but very definite content of the fertility factor X in milk fat.

orange juice daily constitutes an antiscorbutic protection for guinea pigs, it would appear that our dose contained a very great excess of vitamine C. We were met with a relative, even if not complete, failure to restore fertility. It will be noted that only a single case of fertility resulted, though in this instance the young were not of normal weight at the end of the suckling period.\*

TABLE XII

Showing the Reproductive Performance of Rats Reared On the Basic Ration and, After Sterility Was Proven, Fed Also 16 c.c. of Orange Juice Daily

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
B 8410	1 Resorption	2 days before mating	Resorption Resorption
BH 8714	1 Resorption	1 day before mating	Resorption Resorption
W 8329	1 Resorption	11 days before mating	Litter of 5, 5 weaned
B 8752	1 Resorption	1 days before mating	Resorption

Summary: 4 rats used in experiment

On the basic ration:

4 resorptions

0 litters

On the modified basic ration:

5 resorptions

1 litter

Total number of young born..... 5

Total number of young weaned... 5

Average weight of young

at birth ..... 4.6 gms.

Average weight of young

at weaning ..... 29.6 gms.

Percentage loss in mother's weight during lac-

tation ..... 1.6%

g) *Failure to cure with codliver oil.* The very high vitamine A content of codliver oil, together with its possible possession of an additional organic factor curative for rickets (McCollum) led us to add this substance to the basic ration and to attempt similarly to cure instances of proven sterility. The five cases studied in this way are given in Table XIII.

\* Although W 8329 has since had a second litter of five (four weaned), the only two females of the second generation, which have been tested have each resorbed their first litter. They were reared on the basic diet and fed in addition 4 cc. of orange juice daily.



TABLE XIII

Showing the Reproductive Performance of Rats Reared On the Basic Ration and, After Sterility Was Proven, Transferred to This Diet Modified so as to Contain 9% Codliver Oil In Place of 9% Milkfat

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
W 8346	3 Resorptions	14 days after mating	Resorption Resorption Resorption Resorption
B 8347	3 Resorptions	Day of mating	Resorption Resorption
B 8357	1 Resorption	1 day before mating	Resorption Resorption
G 7940	1 Resorption	2 days before mating	Resorption Resorption Resorption at autopsy
W 8097	1 Resorption	2 days before mating	Resorption Resorption

Summary: 5 rats used in experiment

On the basic ration:

9 resorptions

0 litters

On the modified basic rations:

13 resorptions

0 litters

We tested the relative content in vitamine A of the particular lot of codliver oil used by us when compared with the milkfat used, finding it at least eleven times as rich in this substance. The effect on the placental deficiency disease was nil. In another case, detailed below, 24 per cent. of Norwegian codliver oil was present in the basic ration and without effect on fertility.

TABLE XIV

Showing the Reproductive Performance of a Rat Reared On a Modified Basic Diet Containing 24% Milkfat and, After One Gestation, Held On This Diet With the Milkfat Replaced by Codliver Oil (24%)

Designation of Rat	Gestations On the Basic Ration (24% Milkfat)	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet (24% Codliver Oil)
W 8217	1 Litter of 2	7 days after mating	Resorption Resorption

h) *Failure to cure with excess of yeast.* We were led to try an experiment with a high proportion of yeast both from considerations of the qualitative protein change involved and also to settle decisively the effect of superfluous water soluble vitamine B. Our basic ration was made up so as to include 25 per cent. yeast and 25 per cent. casein as follows:

casein .....	25
yeast .....	25
cornstarch .....	22
lard .....	15
salts .....	4
milkfat .....	9

Fed .4-.6 gram fresh dried yeast daily

The case (W 8101) is given below in Table XV. The animal was sacrificed after three resorptions.

TABLE XV

Showing the Reproductive Performance of a Rat Reared On the Basic Diet and, After Sterility Was Proven, Transferred to This Diet Modified So As to Contain 25% Yeast and 25% Casein In Place of 18% Casein

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
W 8101	I Resorption	4 days before mating	Resorption Resorption Resorption

i) *Failure to cure with protein of the basic mixture represented by lactalbumen.\** The possible superiority of lactalbumen

TABLE XVI

Showing the Reproductive Performance of a Rat Reared On the Basic Diet and, After Sterility Was Proven, Transferred to This Diet Modified so as to Contain 18% Lactalbumen In Place of 18% Casein

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
B 7863	I Resorption	22 days before mating	Resorption Resorption Resorption

\* Secured from the Cottage Grove Dairy Manufacturing Laboratory of the Dairy Division of the Bureau of Animal Industry of the United States Department of Agriculture.

over casein (Osborne and Mendel, *Jour. Biol. Chem.*, Vol. 22, 1915, and Vol. 29, 1917) led us to substitute the former protein for the latter. No alleviation of sterility was secured (Table XVI).

j) *Failure to cure with a higher content of protein in the basic ration.* We sought to test the possibility that casein, although an excellent protein for growth, for instance, might on account of its low content in some of the amino acids, be inadequate for reproductive function when present as 18 per cent. We consequently attempted to cure an instance of dietary sterility by modifying our basic ration solely so as to possess 50 per cent. by weight of casein. As the subjoined Table XVII will show, fertility was not affected by this measure.

TABLE XVII

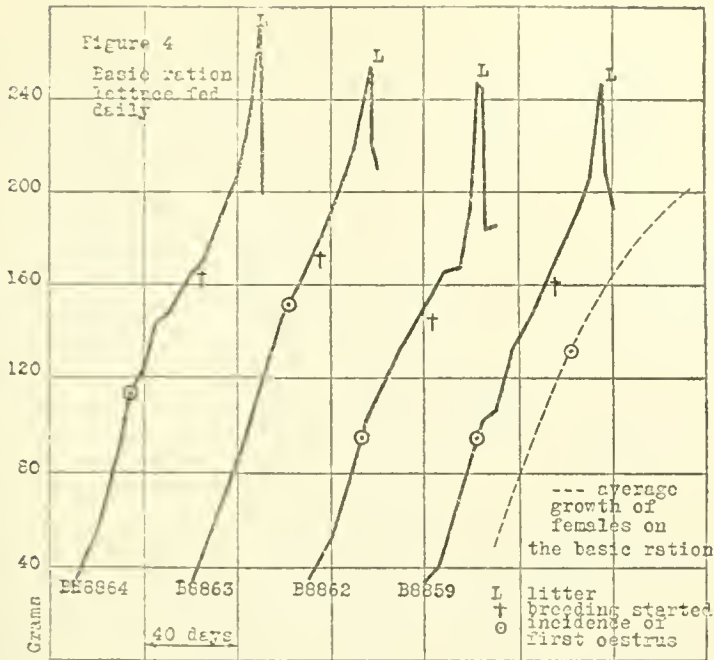
Showing the Reproductive Performance of a Rat Reared On the Basic Diet and, After Sterility Was Proven, Transferred to This Diet Modified So As to Contain 50% Casein In Place of 18% Casein

Designation of Rat	Gestations On the Basic Ration	Time of Modification of Diet with Reference to Next Gestation	Gestations On the Modified Basic Diet
W 8139	1 resorption	4 days before mating	Resorption Resorption

*D. The Prevention of Dietary Sterility by Rearing Animals on the Basic Ration to Which Is Added a Single Natural Foodstuff.\**

Although the possession by a foodstuff of the fertility conferring factor is abundantly shown when fertility is suddenly restored to a sterile animal, it is conceivable that lower amounts of the beneficial factor would escape detection in this way. If the beneficial factor is stored, it is also conceivable that some foods which would not suddenly restore fertility would nevertheless do so if the animal had been held for many weeks or months upon them or had been reared upon them. We have consequently added a single food material to the basic ration and reared young animals in this way from the day of weaning (the

\*The oestrus and ovulation records of the rats reported in this section are given in Appendix Table VI and their reproductive performance in Appendix Table VII.



twenty-first day of life). To be certain of the continuance of the sterility disease in normal controls and, further, to ascertain possible genetic idiosyncrasy of the particular animals used for experimentation, we have in many instances reared littermate sisters on the unmodified basic ration and have tested their reproductive powers in an identical way as was done on the modified ration. Experimental and control rats in this series were bred when 60 days old.

a) *Prevention of sterility with lettuce.* A confirmation of the effects of lettuce as a fertility restorative was readily secured when animals were reared upon the basic regime, having opportunity to consume daily in addition the 40 grams of fresh green leaves which had been proven efficacious with the disabled animals of our curative series. The growth curves of four of these

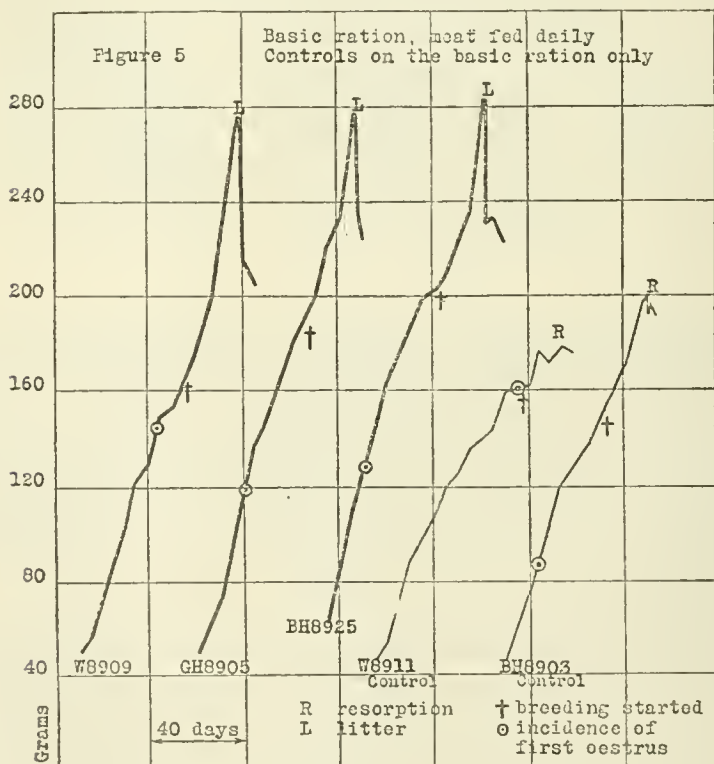
animals are given in Figure 4. Their breeding history is summarized in the following table:

TABLE XVIII

Showing the Reproductive Performance of Rats Reared On the Basic Ration and Also Fed Daily 40 Grams of Fresh Lettuce Leaves

Designation of Rat	Gestations On the Modified Basic Diet
B 8859	Litter of 10, 6 weaned
B 8862	Litter of 9, 6 weaned
B 8863	Litter of 6 (4 dead), 0 weaned
BH 8864	Litter of 11, 6 weaned
W 9059	Litter of 10, 6 weaned
W 9062	Litter of 9, 6 weaned
W 9063	Litter of 9, 4 weaned
W 9061	Litter ? (48 gram drop in weight)

Summary on following page.



## Summary: 8 rats used in experiment

0 resorptions	
1 case of possible litter (drop in weight but no young found)	
7 litters	
Total number of young born.....	64
Number born dead .....	4
Total number of young weaned.....	34
Average weight of young at birth.....	5.3 grams
Average weight of young at weaning.....	28.3 grams
Percentage loss in mother's weight during lactation .....	1.4%

b) *Prevention of sterility with meat.* We attempted three prophylactic cures by offering for a period of an hour daily 10 grams of fresh lean meat (jaw muscles of beef). The animals consumed on the average but 4.7 grams daily. In each instance the first litter cast was an unusually large one. The growth of these animals is given in Figure 5 and a summary of their breeding history in Table XIX.

TABLE XIX

Showing the Reproductive Performance of Rats Reared on the Basic Ration and Also Fed Daily 5 Grams of Raw Beef Muscle, Together With Littermate Controls Held on the Unmodified Basic Ration.

Designation of Rat	Gestations On the Modified Basic Ration	Littermate Controls	Gestations On the Basic Ration
GH 8905	Litter of 9, 5 weaned	BH 8903	Resorption Litter of 1, 0 weaned
W 8909	Litter of 9, (1 dead) 4 weaned	W 8911	Resorption
BH 8925	Litter of 10, 6 weaned		

Summary: 3 experimental rats; 2 littermate controls  
On the modified basic ration:

0 resorptions	
3 litters	
Total number of young born.....	28
Number of young born dead.....	1
Number of young weaned.....	15
Average weight at birth.....	5.1 grams
Average weight at weaning.....	34.8 grams
Percentage gain in mother's weight during lactation .....	7.2%



On the basic ration:

2 resorptions:

1 litter

Total number of young born..... 1  
(lived 3-4 hours only).

Weight at birth ..... 4 grams

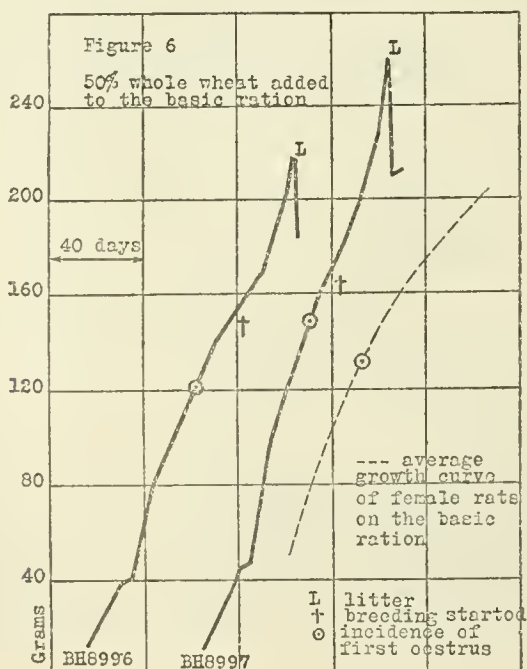
c) *Prevention of sterility with whole wheat.* In two instances we reared animals upon the basic ration to which had been added one-half by weight of whole ground wheat, and the entirely satisfactory result as regards reproduction is shown in the sub-joined table. (The growth curves are presented in Figure 6.)

TABLE XX

Showing the Reproductive Performance of Rats Reared On the Basic Ration Modified So As to Contain 50% Whole Wheat

Designation of Rat	Gestations On the Modified Basic Ration
BH 8996	Litter of 6, 0 weaned (young lived 8-10 days only)
BH 8997	Litter of 8, 6 weaned

Summary on following page.



Summary: 2 rats reared on the modified basic ration

0 resorptions

2 litters

Total number of young born..... 14

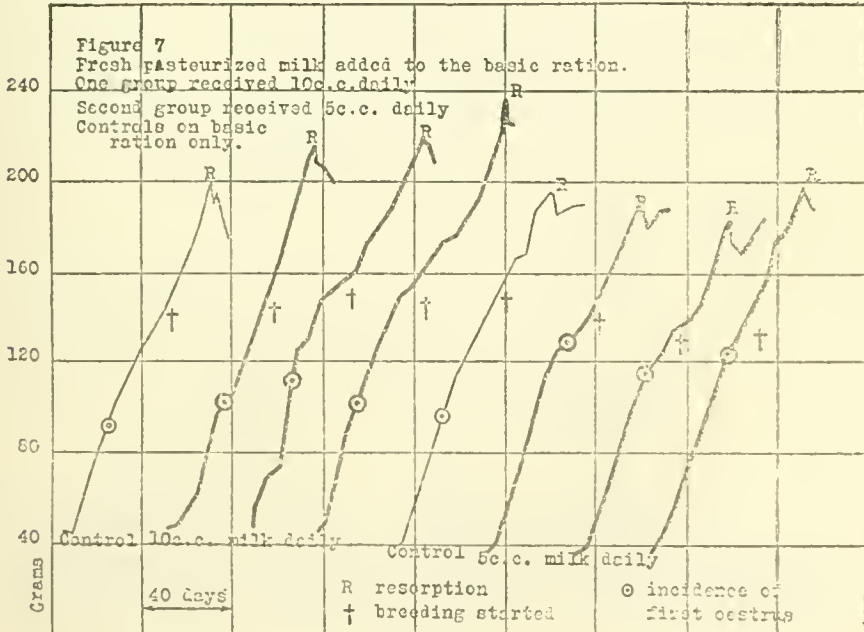
Number of young weaned..... 6

Average weight at birth..... 5.6

Average weight at weaning..... 26.7 gms.

Percentage loss in mother's weight during lactation ..... 9.0%

d) *Attempted prevention of sterility with milk.* When fresh milk was offered daily as was the case with the three animals given in Table IX, throughout the experimental period, no amelioration was obtained of the sterility which is induced by the basic dietary regime. Three animals were given 5 cc. (Table XXI) quantities daily and three were offered 20 cc. (Table XXII), consuming approximately one-half this amount. Sherman has shown that 8 cc. of fresh milk daily contributes enough water soluble vitamine B to serve the rat as the sole source of this factor. Since our animals were fed an additional .6 gm. of fresh dried whole yeast daily, it is evident that we can speak of the second milk group as reared under conditions giving a great excess of B. The growth curves of the animals receiving the



higher milk dose show (Figure 7) slightly but appreciably better growth. The experiment furnishes an instance where improvement in growth is clearly not associated with fertility.

TABLE XXI

Showing the Reproductive Performance of Rats Reared On the Basic Ration and Also Fed Daily 5 c.c. of Fresh Pasteurized Milk, Together With Littermate Control Held On the Unmodified Basic Ration

Designation of Rat	Gestations On the Modified Basic Ration	Littermate Control	Gestations On the Basic Ration
B 8890	Resorption		
G 8891	Resorption		
B 8902	Resorption	W 8899	Resorption

Summary: 3 experimental rats:	1 littermate control:
6 resorptions	1 resorption
0 litters	0 litters

TABLE XXII

Showing the Reproductive Performance of Rats Reared On the Basic Ration and Also Fed Daily 10 c.c. of Fresh Pasteurized Milk, Together With Littermate Control On the Unmodified Basic Ration

Designation of Rat	Gestations On the Modified Basic Ration	Littermate Control	Gestations On the Basic Ration
G 8892	Resorption		
G 8893	Resorption		
GH 8904	Resorption	BH 8903	Resorption
	Resorption		Litter of 1, 0 weaned

Summary: 3 experimental rats:	1 littermate control:
4 resorptions	1 resorption
0 litters	1 litter
	1 young born (lived 3-4 hours only)

As will be shown in the case of the second generation reared on 25 per cent. whole milk powder in the modified basic ration (footnote, Table X), the higher proportion of milk in the diet does not confer fertility until more milkfat is added. It is there-

fore not surprising to find that a basic ration to which 1/3 skim milk powder\* by weight is added does not constitute a diet which permits normal reproduction.

TABLE XXIII

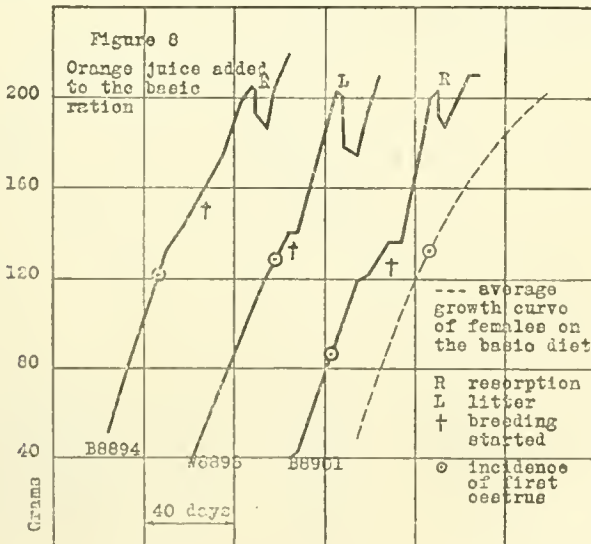
Showing the Reproductive Performance of Rats Reared On the Basic Ration Modified so as to Contain  $\frac{1}{3}$  Skim Milk Powder by Weight, Together With Littermate Controls Reared On the Unmodified Basic Ration

Designation of Rat	Gestations On the Modified Diet	Littermate Control	Gestations On the Basic Ration
W 9074	Resorption		
W 9094	litter? (24 gm. drop in weight)	W 9092	Resorption
W 9095	Resorption	W 9093	Resorption
W 9070	Resorption	W 9072	Resorption

Summary: 4 experimental rats:  
3 resorptions  
1 litter? (no young found)

3 littermate controls:  
3 resorptions  
0 litters

e) *Attempted prevention of sterility with orange juice.* Eight cubic centimeters of orange juice were given daily to three ani-



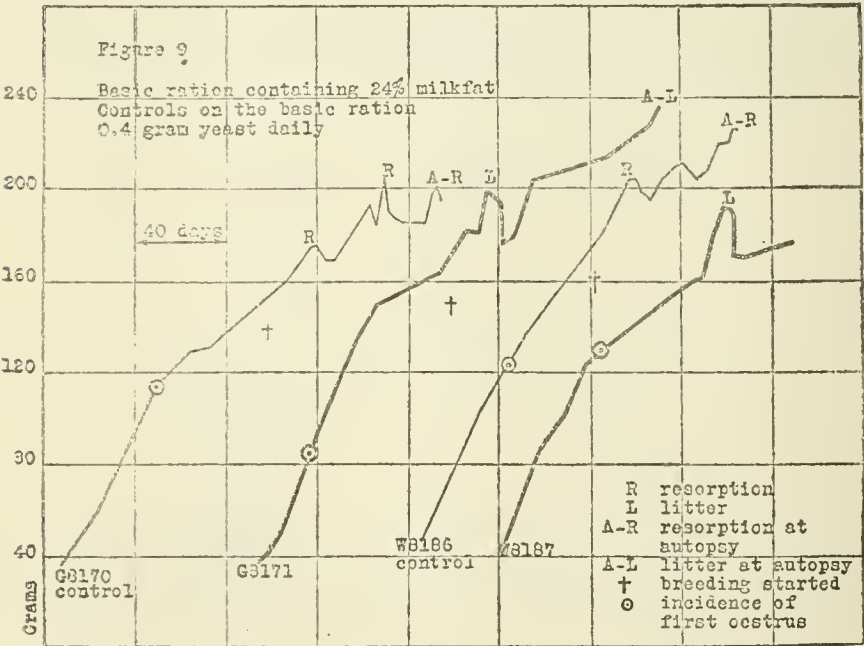
mals from the beginning of the experiment (twenty-third day of life). In one instance a single young was born dead. To date, four resorptions have occurred in the group and there seems to be no evidence but that this history will monotonously repeat itself. (The growth curves are presented in Figure 8.)

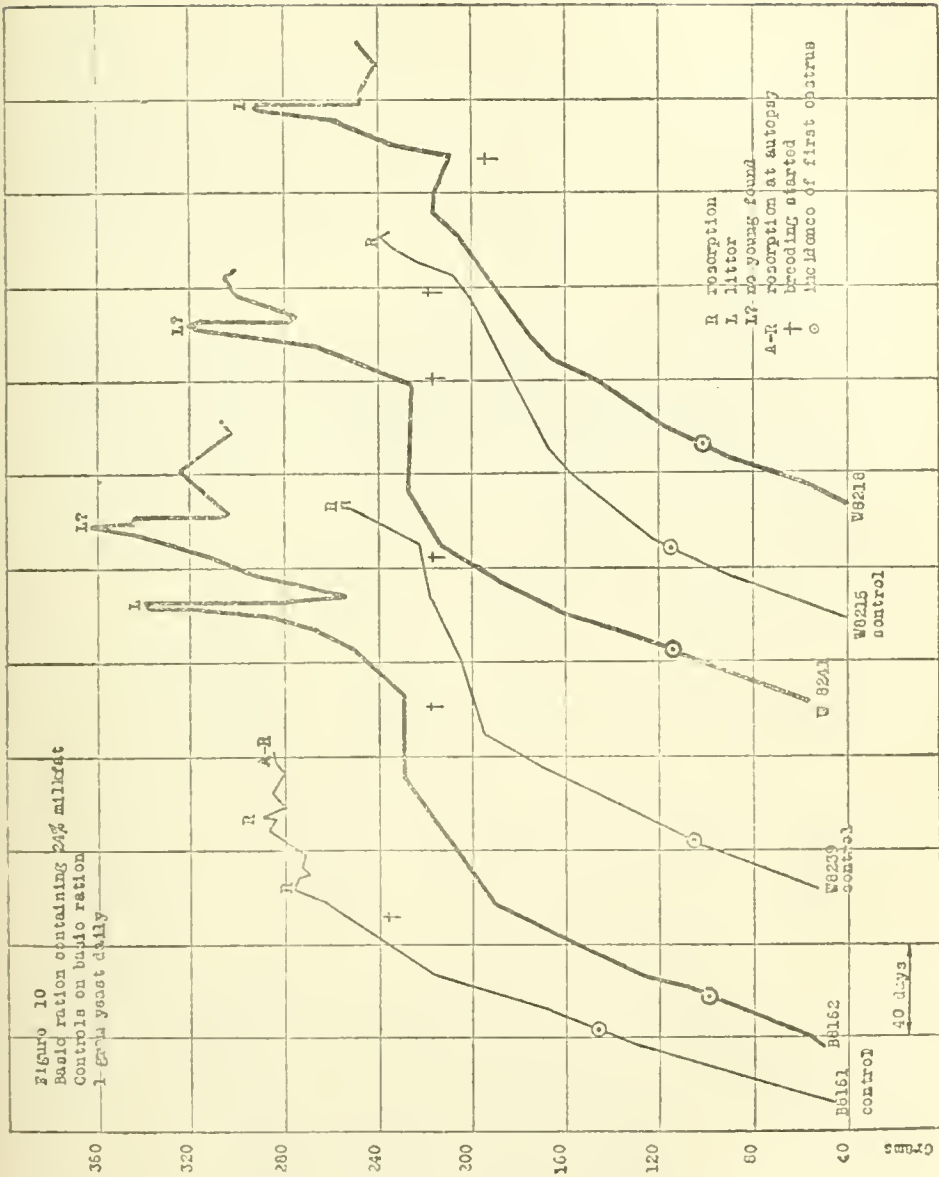
TABLE XXIV

Showing the Reproductive Performance of Rats Reared On the Basic Ration and Also Fed Daily 8 c.c. of Orange Juice, Together with Littermate Control Held On the Unmodified Basic Ration

Designation of Rat	Gestations On the Modified Basic Ration	Littermate Control	Gestations On the Unmodified Basic Ration
B 8894	Resorption Resorption	B 8896	Resorption Resorption
W 8893	Litter of 1 (dead) Resorption		
B 8901	Resorption		

Summary: 3 experimental rats:	1 littermate control:
4 resorptions	2 resorptions
1 litter	0 litter
1 young, born dead	







f) *Prevention of sterility with a higher proportion of milkfat.* We first discovered the effect of large amounts of milkfat when twenty animals were reared on our basic ration but containing 24 per cent. instead of 9 per cent. milkfat. To our surprise these showed a partial fertility. Seven of them would, indeed, seem entirely normal and in only three instances has complete sterility been encountered. Half of these animals received .4 gm. (Table XXV) of fresh dried whole yeast daily as their source of vitamine B and half of them 1 gm. yeast doses (Table XXVI), but significant difference in the reproductive performance of the two groups was not seen. (The growth of some of these animals is given in Figures 9 and 10.)

TABLE XXV

Showing the Reproductive Performance of the First Generation of Rats Reared On the Basic Ration Modified So As to Contain 24% Milkfat In Place of 9% Milkfat and 15% Lard, Together With Littermate Controls Held On the Unmodified Basic Ration. Each Animal Received .4 Gram Yeast Daily

Designation of Rat	Gestations On the Modified Basic Ration	Littermate Control	Gestations On the Basic Ration
W 8164	Litter of 1 (dead) Litter of 3, 3 weaned	G 8170	Resorption Resorption Resorption at autopsy
G 8171	Litter of 4, 1 weaned Litter of 2 at autopsy		
W 8187	Litter of 4, 2 weaned		
W 8214	Resorption	W 8186	Resorption Resorption at autopsy
W 8217	Litter of 2, 0 weaned		
W 8230	Litter of 2 (dead)		
W 8240	Litter of 6, 0 weaned Resorption Litter of 4 (1 dead), 3 weaned	W 8246	Resorption Resorption
W 8248	Litter of 2, 0 weaned Litter of 8 at autopsy		
GH 8254	Litter of 2 (1 dead), 0 weaned		
B 8321	Litter of 3, 0 weaned Litter of 4, 3 weaned Litter of 5, 4 weaned	GH 8252	Resorption

Summary: 10 experimental rats	4 littermate controls
2 resorptions	8 resorptions
15 litters	0 litters
Total number of young born.. 42	
Number of young born dead.... 5	
Number of living foetuses at autopsy ..... 10	
Number of young weaned..... 16	
Average weight at birth.. 5.1 gms.	
Average weight at weaning ..... 20.6 gms.	
Percentage gain in mother's weight during lactation ..... 1.9%	

TABLE XXVI

Showing the Reproductive Performance of the First Generation of Rats Reared On the Basic Ration Modified So As to Contain 24% Milkfat In Place of 9% Milkfat and 15% Lard, Together With Littermate Controls Held On the Unmodified Basic Ration. Each Animal Received 1 Gram Yeast Daily

Designation of Rat	Gestations On the Modified Basic Ration	Littermate Control	Gestations On the Basic Ration
B 8162	Litter of 7, 6 weaned Litter ? (35 gram drop in weight)	B 8161	Resorption Resorption Resorption at autopsy
G 8173	Litter of 4, 0 weaned		
W 8218	Litter of 7, 6 weaned	W 8215	Resorption
W 8220	Litter of 4, 0 weaned Litter of 8, 5 weaned		
W 8231	Litter? (16 gram drop in weight)		
W 8241	Litter? (35 gram drop in weight)	W 8239	Resorption
W 8244	Litter of 7, 0 weaned Resorption		
W 8249	Litter of 1 (born dead) Litter of 3 at autopsy	W 8247	Resorption Resorption Resorption Resorption at autopsy
B 8255	Litter of 2 (1 born dead), 0 weaned	GH 8253	Resorption
B 8322	Litter of 4, 4 weaned Litter of 7 (3 born dead), 4 weaned Litter of 2, 0 weaned		

Summary: 10 experimental rats:                    5 littermate controls  
                  1 resorption    10 resorptions  
                  3 cases of possible    0 litters  
                  litters (drop in  
                  weight but no lit-  
                  ter found)

12 litters

Total number of young born.... 53

Number of young born dead.... 5

Number of living foetuses at  
 autopsy ..... 3

Number of young weaned..... 25

Average weight at birth.. 5.0 gms.

Average weight at wean-  
 ing ..... 25.6 gms.

Percentage gain in moth-  
 er's weight during lac-  
 tation ..... 1.1%

Another test of the potency of milkfat was made by continuing to rear some of the female young produced from such pregnancies upon the same ration as had conferred fertility upon the mother (i. e., the basic ration modified so as to contain 24 per cent. milkfat), and the results to date amply confirm the striking first generation results. (The growth of these animals is shown in Figure 11.)

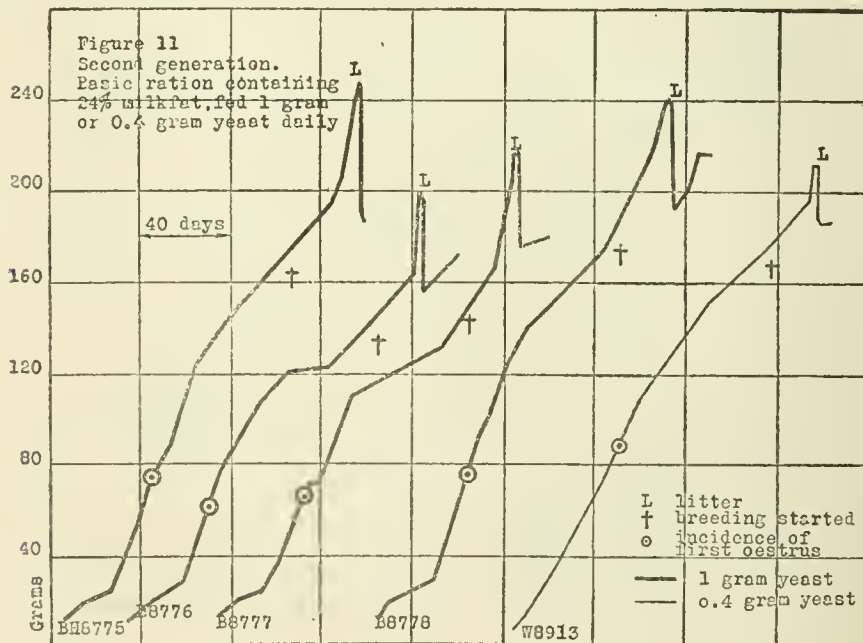


TABLE XXVII

Showing the Reproductive Performance of the Second Generation of Rats Reared On the Basic Ration Modified So As to Contain 24% Milkfat and Fed Daily Yeast Doses of .4 Gram or 1 Gram as Noted

Designation of Rat	Gestations On the Modified Basic Rations
Litter of W 8187: .4 gram yeast  W 8913	Litter of 4, 0 weaned (young lived 5 days only) Average weight at birth: 4.5 grams
Litter of B 8322: 1 gram yeast  BH 8775 B 8776 B 8777  B 8778	Litter of 9, 5 weaned Litter of 6 (1 dead), 4 weaned Litter of 8 (1 dead), 0 weaned Litter of 9, 5 weaned Litter of 9, 0 weaned

Summary: 4 second generation experimental rats (1 gram yeast dose)  
5 litters

Total number of young born.....	41
Number of young born dead.....	2
Number of young weaned.....	14
Average weight at birth.....	4.9 grams
Average weight at weaning.....	24.1 grams
Percentage gain in mother's weight during lactation .....	8.7%

g) *Attempted prevention of sterility with codliver oil.* To date three animals have been reared upon the basic ration modified so as to have, instead of milkfat, 9 per cent. codliver oil. Indications of a partial fertility appear here although the placental disease, as evidenced by complete resorption of the young, has already shown itself in the second gestation of the two animals to whom at first living young were born. In one of these cases (W 8906) only a single young was born, an occasional occurrence with animals on the basic diet and, oddly enough, actually occurring in the second gestation of the littermate control (BH 8903) of this animal, maintained on the basic diet, so that fertility could not be said to be influenced by codliver oil in this case.

TABLE XXVIII

Showing the Reproductive Performance of Rats Reared on the Basic Ration Modified So As to Contain 9% Codliver Oil In Place of 9% Milkfat, Together With Littermate Controls Held On the Unmodified Basic Ration

Designation of Rat	Gestations On the Modified Basic Ration	Littermate Control	Gestations On the Basic Ration
W 8906	Litter of 1, 0 weaned Resorption	BH 8903	Resorption Litter of 1, 0 weaned
W 8912	Resorption	W 8911	Resorption
G 8934	Litter of 4, 0 weaned Resorption	W 8937	Resorption Resorption

Summary: 3 experimental rats:

3 resorptions

2 litters

Total number of young born... 5

Young lived 1-3 days only.

Average weight at birth: 4.8 gms.

3 littermate controls:

4 resorptions

1 litter

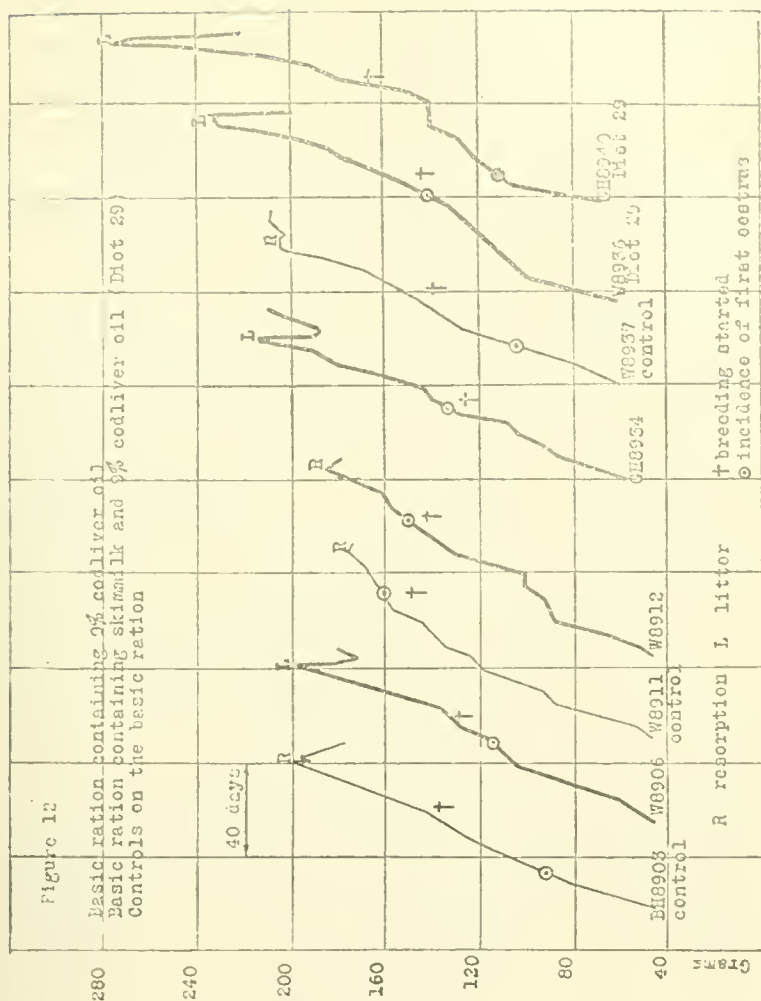
Total number of young born 1

Young lived 3-4 hours only.

Average weight at birth: 4 gms.

We happened to use a modified basic ration which contained 1/3 skim milk powder by weight and 9 per cent. codliver oil (Diet 29), securing three gestations where two or three young were carried to term and born dead and one where two defective young lived a single day. (The growth of these animals is given in Figure 12.) We are at a loss to explain the lessened severity of the disorder.

h) *Attempted prevention of sterility with excess of yeast.* As has been stated, the daily dose (.2—.4 gram) of dried Harris yeast adequate for growth was possibly not optimal for ovulation and it would seem possible that a still higher amount of B might be needed for complete reproductive performance. However, we have found that increasing the daily yeast feedings to 1 gram amounts, which is almost as much as a rat will consume when the yeast is fed separately, has no effect upon reproduction. Tables XXV and XXVI summarize the behavior of a part of the experimental group so treated, which also served as controls for the 24 per cent. milkfat diet. (The growth of some of these animals is given in Figures 9, 10 and 13.) It is clear that fertility does not result until the milkfat is raised to 24 per cent. of the diet and that even then the rats receiving 1 gm. yeast exhibit no better



performance than those on .4 gm. Table XXIX shows the complete sterility of the remainder of the two yeast groups when receiving 9 per cent. milkfat in the diet.



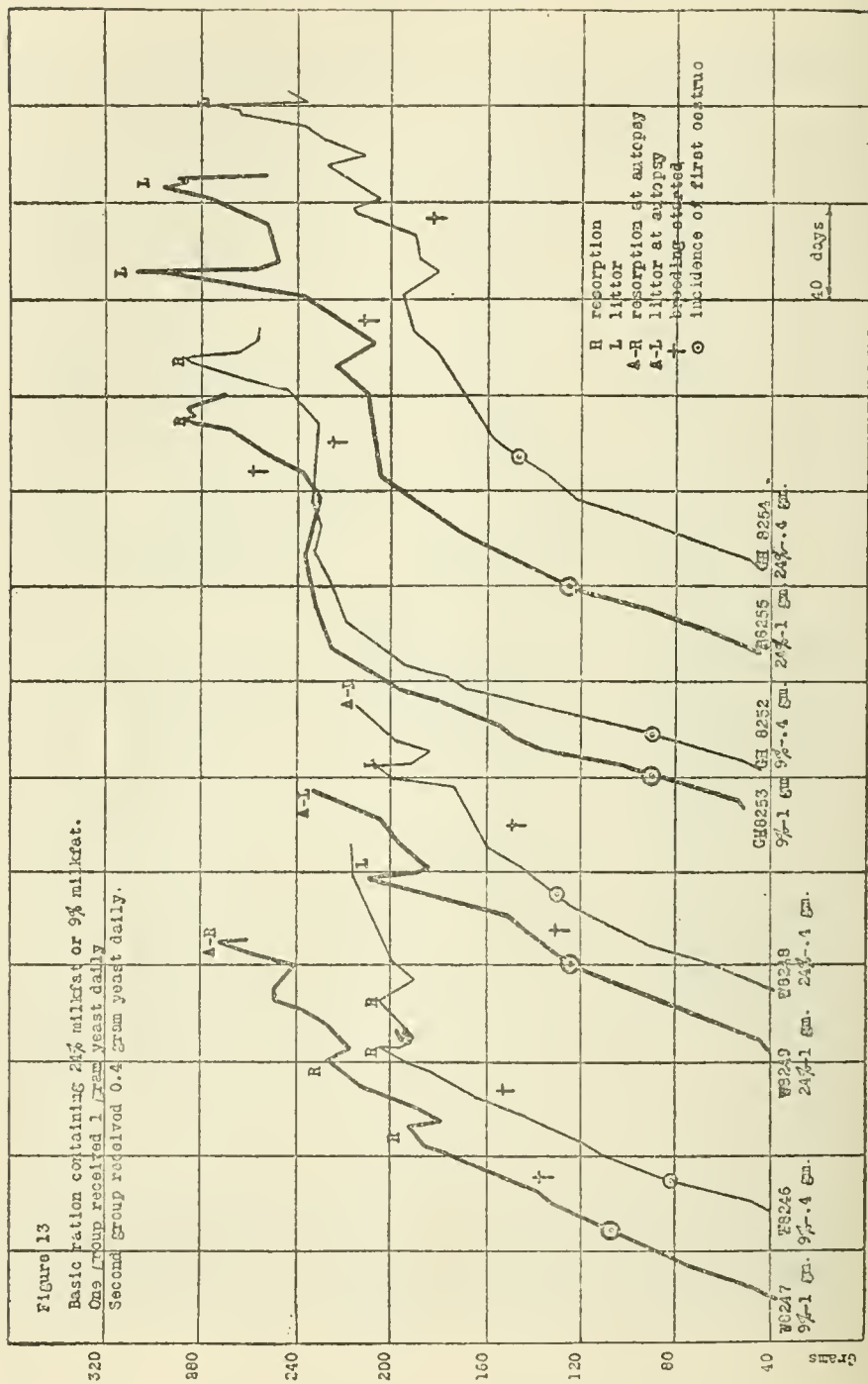


TABLE XXIX

Showing the Reproductive Performance of Rats reared on the Basic Ration and Fed Daily 1 Gram of Dried Yeast, Together With Littermate Controls Fed .4 Gram Yeast Daily

Designation of Rat	Gestations On Basic Ration and 1 Gram Yeast Dose	Littermate Control	Gestations On the Basic Ration and .4 Gram Yeast Dose
W 8165	Resorption Resorption at autopsy		
W 8168	Resorption Resorption at autopsy	W 8167	Resorption Resorption Resorption at autopsy
W 8174	Resorption	W 8172	Resorption Resorption
W 8188	Resorption at autopsy		
B 8195	Resorption Resorption Litter of 7 at autopsy		
W 8224	Resorption		
W 8234	Resorption	W 8233	Resorption Resorption

Summary: 7 rats fed 1 gram yeast  
10 resorptions  
1 litter of 7 living  
foetuses at autopsy.

3 rats fed 0.4 gram yeast  
7 resorptions  
0 litters

TABLE XXX

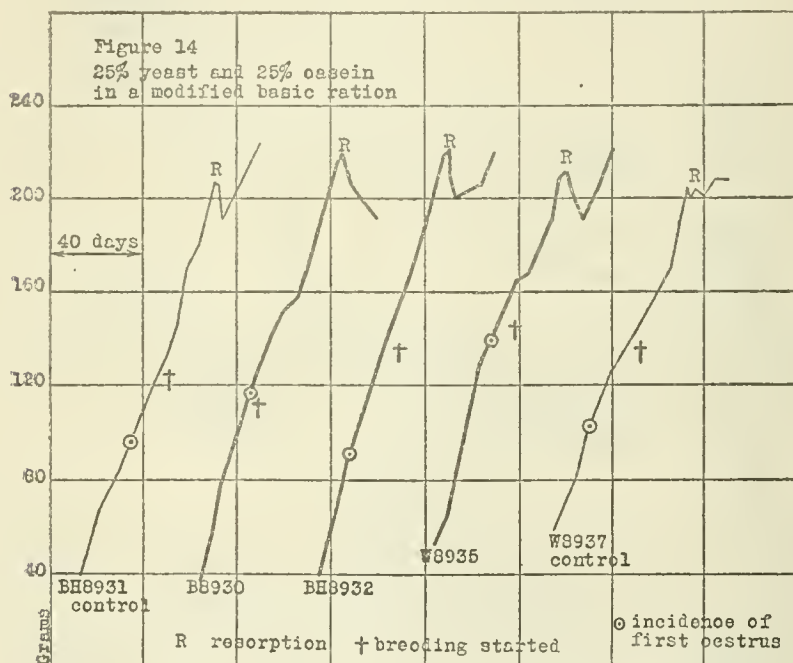
Showing the Reproductive Performance of Rats Reared On the Basic Ration Modified So As to Contain 25% Yeast and 25% Casein, Together With Littermate Controls Held On the Unmodified Basic Ration

Designation of Rat	Gestations On the Modified Basic Diet	Littermate Control	Gestations On the Basic Ration
B 8930	Resorption		
BH 8932	Resorption	BH 8931	Resorption
W 8935	Resorption Resorption	W 8937	Resorption Resorption

Summary: 3 experimental rats  
4 resorptions  
0 litters

2 littermate controls  
3 resorptions  
0 litters

The additional protein and high vitamine B given when the basic ration contained at the same time 25 per cent. of yeast and 25 per cent. of casein and which led us to try this as a curative substance, also led us to try its effect upon animals reared on such a regime. Table XXX shows that the three individuals treated in this way did not exhibit any discernible improvement, four resorptions having been recorded to date. (The growth of these animals is given in Figure 14.)



i) *Attempted prevention of sterility with protein of the basic mixture represented by lactalbumen.*

A single attempt was made to discover whether there were in lactalbumen, properties superior to casein when animals were reared on the basic diet in which this protein replaced casein. Fertility was not produced. (The growth of this animal is given in Figure 15.)

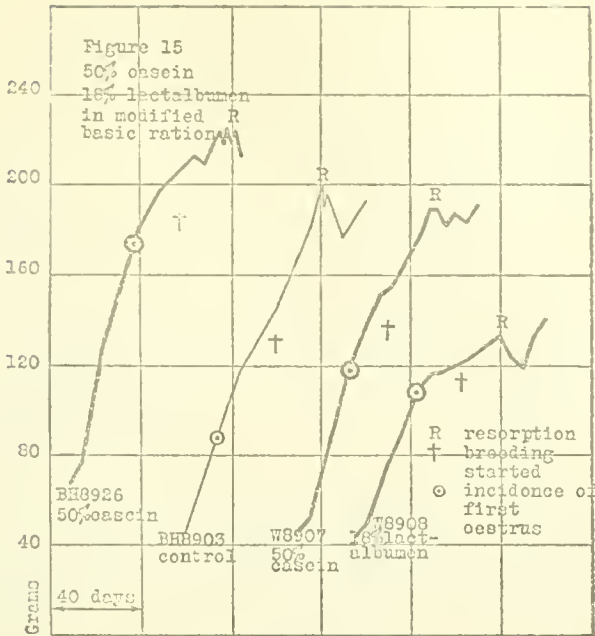


TABLE XXXI

Showing the Reproductive Performance of a Rat Reared On the Basic Ration Modified So As to Contain 18% Lactalbumen In Place of 18% Casein, Together With Littermate Control Held On the Unmodified Basic Ration

Designation of Rat	Gestations On the Modified Basic Ration	Littermate Control	Gestations On the Basic Ration
W 8908	Resorption Resorption at autopsy	BH 8903	Resorption Litter of 10 weaned

Weight at birth: 4 grams.

Young lived 3 to 4 hours only.

j) *Attempted prevention of sterility with higher protein.* Two animals have been reared upon our basic diet with 50 per cent. instead of 18 per cent. casein. To date three resorptions have occurred and there is no intimation that fertility can be produced in this way. (The growth of these animals is shown in Figure 15.)

k) *Attempted prevention of sterility with addition of small quantities of cystine to the basic ration.* Since cystine seems the

TABLE XXXII

Showing the Reproductive Performance of Rats Reared On the Basic Ration Modified So As to Contain 50% Casein, Together With Littermate Control Held On the Unmodified Basic Ration

Designation of Rat	Gestations On Modified Basic Ration	Littermate Control	Gestations On the Basic Ration
W 8907	Resorption Resorption	BH 8903	Resorption Litter of 1 0 weaned
BH 8926	Resorption		

Summary: 2 experimental rats  
3 resorptions  
0 litters

1 littermate control  
1 resorption  
Litter of 1 young,  
lived 3-4 hours only  
Weight at birth: 4 grams

only important amino acid not contained in adequate amount in the protein casein, we have added cystine\* to the basic ration, otherwise unchanged in its formula. The cystine was in two cases added in amount sufficient to give the same proportion of cystine in the diet as would be present if 18 per cent. gliadin (containing .5 per cent. cystine) replaced the 18 per cent. casein, or 0.09 gram cystine in 100 grams of the basic ration. In the third case, what would seem to be an excess of cystine (2 per cent. of the protein present) was added, namely, 0.36 gram to 100 grams of the ration. As is seen in Table XXXIII, there has been no improvement in the behavior of the experimental rats during the first gestation, all three showing one resorption apiece.

TABLE XXXIII

Showing the Reproductive Performance of Rats Reared On the Basic Ration to Which Is Added Cystine In Two Amounts, 0.5% of the Protein and 2% of the Protein, Respectively, Together With Littermate Controls Reared On the Unmodified Basic Ration

Designation of Rat		Gestations On the Modified Diet	Littermate Control	Gestations On the Basic Ration
Cystine as 0.5% of protein weight:	BH 9125	Resorption	B 9124	Resorption
	BH 9127	Resorption	BH 9126	Resorption
Cystine as 2% of protein weight:	W 9129	Resorption	BH 9128	Resorption

\* We are greatly indebted to Dr. C. L. A. Schmidt of the University of California for the purified cystine used in these experiments.

Summary: 3 experimental rats	3 littermate controls
3 resorptions	3 resorptions
0 litters	0 litters

*E. The Dietary Cause of Sterility on the Basic Ration.*

1. *Possible deficiency of the basic mixture in known substances.* We are not at liberty to assume the existence of a peculiar dietary need for reproduction or the existence of a vitamine essential for this process before exhausting our search into the possible inadequacies of our diet in well known nutritive factors. It is entirely conceivable that the special drain put upon the organism in its provision for the rapidly developing young may demand a somewhat different quantity or quality of the three fundamental foodstuffs, of salts or of the three well known vitamines. We propose to discuss these possibilities.

a) *Protein inadequacy.* We sought to discover whether an increase in casein over the 18 per cent. present in our basic diet would confer fertility, but this did not appear to be the case. Furthermore, lack of improvement when yeast protein or lactalbumen were employed showed that these two other proteins at any rate have no advantage over casein. Our evidence, indeed, points to their inferiority. Since chemical analyses have repeatedly indicated cystine deficiency in casein, we have added this amino acid to the protein. However, since the placental disorder was not ameliorated in this way, it appears extremely unlikely that protein quality is the responsible factor as to whether the gestation is normal, or, on the other hand, must inevitably be resorbed. When, in conjunction with this, we view the fact that a high quota of milkfat restores fertility, it would appear certain that we can entirely relieve the protein moiety of any essential complicity in fertility or sterility results.

b) *Proportions of carbohydrate or fat.* Since the discovery by Osborne and Mendel that rats may be reared successfully upon diets almost free of appreciable quantities of fat or preformed carbohydrate respectively, we have carefully repeated the experiments necessary to substantiate their contention and have already pointed out (*J. Met. Res.*, March, 1922) that when the protein quota is neither excessively high nor low, such animals do not grow differently from their controls on the basic ration. With casein as 23 per cent. or even as 50 per cent. of the ration, neither fat nor preformed carbohydrate is essential in the diet as deter-



mined by growth and, furthermore, by the far more sensitive test of gonadal physiology (time of first oestrus and oestrous rhythm), as we can state. It is of interest to know that the reproductive history of these groups was not markedly abnormal when compared with their littermate sister controls. Our tests of reproduction were carried out during the time interval when we secured a partial fertility in animals on the basic ration.

Interest and probably significance attaches to the fact that the animals reared on the fat free modifications of the basic diet were more fertile than their controls (as shown by the group fertility per cent. and placental index, although the implantation per cent. was low). It would appear proper for us to look upon the higher fertility of these groups when compared with the carbohydrate free groups (whose diet contains fat) as due to the favorable effects of a new substance added to their ration rather than to the highly questionable benefit of fat absence in their food. The single new substance added was .35 gram daily of powder made from the carefully dried leaves of alfalfa, administered to satisfy the vitamine A requirement since, of course, less fat would be introduced in this way than with milkfat. The restoration of placental function which we have effected by feeding lettuce leaves with the basic ration would appear to justify us in assigning the otherwise perplexing improvement on the fat free diets to some desirable element introduced by the green leaf.\*

It is further to be especially noted that although animals maintained on the basic ration are invariably sterile in the second generation, we secured 15 litters from 15 second generation females held upon fat free basic diets to which alfalfa leaf powder was added, while only 9 resorptions occurred in this generation, and there has been 1 litter to date from the third generation, on this fat free ration (casein 23 per cent.), with no resorptions.

c) *Possible salt inadequacy.* Inasmuch as the inorganic salts which we added to our ration, although calculated to take care of the major nutritional salt needs, contained only seven elements: Na, Cl, Mg, S, P, Ca, Fe, it is possible that certain traces present in the ash from natural foods might constitute the element, or elements, so essential for placental function. We refer to iodine, manganese, aluminum and flourine particularly.

\* We have added evidence of this in the birth of a litter from each of two rats reared on the basic ration and fed .35 gram alfalfa leaves daily in addition to the yeast dosage. We desire to thank Prof. Geo. W. Hendry of the Agronomy Division of the College of Agriculture for his many courtesies in supplying carefully dried alfalfa samples.

It is impossible at the present time to furnish an exhaustive test of this particular point, but we would point out the unlikelihood that we can explain the fertility of animals reared on a diet where milkfat constituted 24 per cent. of the ration by recourse to a belief in salt improvement. In this connection, it may be remembered that when whole milk powder constituted one-third by weight of the ration and milk salts were hence introduced, fertility results were not secured. It is further a rather surprising fact that the entire salt quota, which we have always added to the basic ration, may be dispensed with and yet fertility result. During the time that a partial fertility was shown by all animals on the basic ration, there occurred instances of normal litters being born to animals which were reared on the basic ration totally devoid of salts, although we would not wish this experience to indicate a disbelief of possible salt relations in fertility. We would emphasize merely that our peculiar sterility deficiency disease was not produced by lowering the salt content of the diet.

2. *Components of curative foods, other than vitamins, which may be responsible for the cure.\**

The efficacy both of a green leaf and of meat in restoring fertility inevitably suggests the possibility that we may be dealing with the introduction of the most important coloring matter of plants and animals, i. e. chlorophyll and hemoglobin and, hence, with the needed pyrrol ring, which the body is unable to synthesize. However, we have obtained only negative results by adding commercial preparations of chlorophyll to the basic ration. Two rats fed daily 0.1 gram of Phyllosan\*\* (containing 40 per cent. by weight of chlorophyll) have shown one resorption apiece. Further, if we consider the potency of a high proportion of milkfat, another pigment, carotin, must be concerned in fertility results. The explanation that our beneficial substance is related to pigment metabolism seems remote.

---

\* We have had no experience which would bear on the cure of sterility by means of agar in the diet as roughage, reported by Mitchell. Our autopsy findings did not indicate intestinal stasis; they did disclose a specific trouble with foetus and placenta, starting at an early period in gestation and the cures we have secured with such a substance as milkfat (consistency of diet unchanged), make the author's explanation of her results seem inadequate.

\*\* The preparation used was Merck's PHYLLOSAN, made by the Swiss Serum and Vaccine Institute, Berne, Switzerland. For this we wish to express our thanks to Frieda S. Robbins.

### 3. *Segregation of the fertility conferring factor X from the vitamins hitherto known.*

a) *Segregation from fat soluble vitamine A.* The occurrence of fertility in animals reared upon our basic ration in which the proportion of milkfat was raised to 24 per cent. naturally suggests that we may be concerned with effects secured from a necessary excess of vitamine A. It is entirely conceivable that the reproductive function might call for a greater amount of this substance than is necessary for the prevention of xerophthalmia and other deficiency disorders due to lack of A and in excess of the demands of bodily growth. It may be pointed out that the 9 per cent. milkfat present in the basic ration does as a matter of fact constitute such an excess of vitamine A. We have, in fact, on several occasions happened to rear rats to somewhat beyond the one-hundredth day of life on the basic ration but containing merely 2 per cent. milkfat instead of 9 per cent. and have seen that normal growth and ovulation resulted. More pertinent is the fact that we have repeatedly secured normal reproduction and hence placental function in animals maintained with far less vitamine A than that present in the basic ration, providing only that the diet contained the factor X. We have shown that this factor is present in the cereals. It was shown to be present in whole wheat and in wheat embryo and is evidently present in oats. On using a dietary consisting essentially of oats, gelatine, casein and dextrin and with a content merely of 1 per cent. milkfat, i. e.,

#### Diet 2 (McCollum)

rolled oats .....	40.0
Gelatin .....	10.0
casein .....	5.0
salt mixture (185).....	3.7
dextrin .....	40.3
milkfat .....	1.0

approximately normal growth and normal ovulation occurs, at least until the second generation. These animals were also not greatly embarrassed in their reproductive powers. The specific placental disease found when the factor X is absent did not develop. Ample biological proof that in these cases fat soluble vitamine A is dangerously low is seen when we investigate the second generation of animals maintained on this ration. On about the one-hundredth day of life in the second generation of animals maintained on the ration, there occurs the characteristic

abnormality of the ovulation cycle which we have shown to be a pathognomonic sign of A deficiency (Evans and Bishop, *J. Met. Res.*, March, 1922). If the 1 per cent. milkfat be removed from the dietary, the sign develops almost immediately in all sexually mature animals whether or not they have reached the hundredth day of life. This experiment was conducted by us in six cases of second generation rats on this diet.

Essentially the same type of result has been secured in our studies on fertility with wheat as the cereal present and with fat soluble vitamin very low. Sherman has shown that whole wheat and whole milk in the proper proportions constitute an adequate diet for both reproduction and growth. By eliminating the milkfat from the milk quota we may investigate whether interference with reproduction can be produced and, furthermore, whether that interference takes the form of our specific sterility deficiency disease. It has been possible for us to show clearly our new characteristic sign of A deficiency with diets constituted essentially by two-thirds whole wheat and one-third skim milk powder. It has also been possible for us to secure fertility in such animals even during the time that the undoubted distress signals were given from the lack of A.

Although the reproductive powers were hindered, the sterility disease itself did not develop when animals were maintained solely on milk, whether this be fresh or in the form of whole milk powder, and we have been able to secure reproduction in such animals. Our work with groups of rats reared on the addition of whole milk powder or merely of milkfat to the basic ration has made it seem most probable that the factor X is confined to the fat quota of milk, and the argument which has just been developed would lead us to state that the beneficial effect of a very high percentage of milkfat seems preferably explained by its possession of a definite, though low quota of the fertility conferring factor X, rather than by its augmentation of the fat soluble vitamin A.

Although definite slight effects on fertility were secured with codliver oil, this substance is on the whole ineffectual to aid foetus and placenta. There can be no question about its superior content in vitamin A, a fact which we ourselves established for the particular sample of codliver oil used by us in our work. One drop daily of this substance saved the life of several animals in distress and final decline from A deficiency disease. It is evident



that the 9 per cent. of this particular codliver oil present in the basic ration instead of milkfat constituted a very great, indeed very abnormal, augmentation in the vitamine A resources. Yet the animals reared upon this regime as a rule continued to exhibit sterility.

It appears therefore, that while the beneficial factor X is distributed so as to be high in some substances which are also high in A (lettuce, alfalfa), it is very low in other substances high in A (whole milk, milkfat, codliver oil) and high in some substances low in A (lean meat, wheat). There would thus seem to be no doubt about its segregation from A.

b) *Segregation from water soluble vitamine B.* We have already developed the argument which leads us to believe that sterility in animals maintained on the basic ration does not result because water soluble vitamine B is low. We know that in all our work where .6 gram of fresh dried yeast (Harris) was administered to animals in daily doses, an abundance of B was present. Furthermore, when twice or more of this dose of yeast is administered, the sterility disease continues, and this is the case where, in addition to the yeast, another source of vitamine B was introduced as in the animals consuming about 10 cc. of fresh milk daily. Osborne and Mendel have shown that when yeast is fed in very high amounts it appears to exert some toxic effect upon the organs of generation but they have not indicated that this is the case when it is fed in amounts merely necessary to give an adequate source for vitamine B; nor do we believe that in the latter case we could be dealing with specific yeast toxicity. The curative foods could hardly be assumed to detoxify. Yet assuming that they do, it would be most probable that definite amounts of the detoxifying food would offset definite amounts of yeast. Could we secure barely enough of a fertility conferring food to but partially restore reproduction with .4 gram yeast in the basic ration, then the same diet with 1 gram of yeast should be but half as efficient. It so happens that we have performed this experiment in the work with 24 per cent. milkfat (Tables XXV and XXVI) and significant differences in reproduction in the two groups in accordance with the amount of yeast present were not secured. Fertility when wheat germ is used as a source of B results, in our opinion, not because more B is thus introduced, but because wheat germ is also rich in the factor X.

It would appear that fertility effects secured with large amounts of milkfat seem decisive against the complicity of B in this result, for even in this amount of milkfat it is certain that there was not as great an increase in the B quota of the diet as we secured in other ways which did not cure the reproductive disorder.

c) *Segregation from the antiscorbutic C.* The fertility effect secured with fresh green leaves of lettuce and with dried alfalfa leaves demands inquiry into the certainty of segregation of the new dietary factor from the antiscorbutic C. It would appear that ample proof of such segregation is afforded by the curative effects secured with cereals (rolled oats, ground whole wheat and wheat germ), for the cereals are, of course, notably deficient in C. More important perhaps, have been our ineffectual attempts to improve fertility with abundant fresh and undoubtedly potent orange juice.

d) *Segregation from the antirachitic D.* It would also appear unquestionable that our substance is distinct from the antirachitic D, which McCollum has shown is so abundant in codliver oil. In our work codliver oil could not be described as potent for the restoration or maintenance of fertility.

Our work with yeast indicates a segregation of our new substance from the so-called "vitamine D" of Funk. This substance is not known to us as regards its distribution in the common foods, or, indeed, its significance for mammalian physiology, since it has only been brought into relation with the growth of yeast itself.

We have undertaken further experiments designed to characterize the solubility, temperature lability and other peculiarities of the beneficial factor. Its presence in our almost waterfree milkfat is presumptive of its solubility in fat, and its occurrence in dried whole milk powder and in meat boiled for an hour, when constituting the sole item of diet, argues for a considerable degree of resistance to temperature. The correspondence of results obtained with our curative and our prophylactic experiments and some preliminary results as shown in Table XIV seem to indicate that the factor, in this respect like water soluble vitamine B, cannot to any considerable extent be stored by the body. We are also extending our examination of the presence of the unknown factor in a considerable series of human and animal foods.



*F. Summary.*

1. The classic "basic ration" (casein-lard-cornstarch mixture) used for studies on growth fails to give normal fertility in a large proportion of female rats reared upon this diet.

2. The sterility produced does not interfere with the early steps in gestation. The ovulation and implantation incidence is normal, but most of the implantations are resorbed (from 80 per cent. to 100 per cent.). In two clear instances of proven sterility, fertility was restored by a beneficial food (leaves, high milkfat) given after ovulation had occurred. This contributes to the unlikelihood that the germ cells themselves are diseased, but this is not impossible.

3. The sterility may either be prevented or cured, after its appearance, in any individual case by the addition of certain natural foodstuffs to the basic ration.

4. Lettuce, meat, whole wheat, wheat germ, rolled oats, dried alfalfa and large quantities of milkfat are such curative substances.

5. Whole milk, fresh or dried, codliver oil, orange juice and yeast fail to act as curative agents when added to the basic diet.

6. The sterility was not influenced by certain changes in the quality or quantity of the protein in the basic ration.

7. There is no evidence that the salt content of the basic ration is at fault.

8. Varying the proportions of fat and carbohydrate in the ration has not influenced the specific sterility deficiency disease.

9. The unknown fertility conferring factor (X), shown to be present in lettuce, meat, wheat, oats, alfalfa and milkfat, cannot be identified with the known vitamins A, B, C, or D.

10. The factor X, does not seem to be stored for long periods in the animal body, and is resistant to ordinary cooking temperatures.

Submitted Jan. 23, 1923.

## BIBLIOGRAPHY

- Davey, A. J. Determination of the minimum doses of some citrus fruit juices which will protect a guinea-pig from scurvy, together with some observations on the preservation of such juices. *Biochem. J.* 15, 1921, 83.
- Drummond, J. C. Researches on the fat-soluble accessory substance. II. Observations on its rôle in nutrition and influence on fat metabolism. *Biochem. J.*, 13, 1919, 95.
- Evans, H. M. and Bishop, K. Scott. On the relations between fertility and nutrition. I. The ovulation rhythm in the rat on a standard nutritional regime. *J. Met. Res.*, 1, 1922, 319.
- Evans, H. M. and Bishop, K. Scott. On the relations between fertility and nutrition. II. The ovulation rhythm in the rat on inadequate nutritional regimes. *Ibid.*, 335.
- Kennedy, C. and Palmer, L. S. Yeast as a source of vitamine B for the growth of rats. *J. Biol. Chem.*, 54, 1922, 217.
- Long, J. A. and Evans, H. M. The oestrus cycle in the rat and its associated phenomena. *Memoirs of the Univ. of Calif.*, vi, *Univ. of Calif. Press*, Berkeley, 1922, 148 pp., 11 pl., 4°.
- Mitchell, H. S. Reproduction on "synthetic" diets when purified agar is added to the mixture. *Amer. J. Phys.*, 62, 1922, 557.
- Palmer, L. S. and Kennedy, C. The relation of plant carotinoids to growth and reproduction of albino rats. *J. Biol. Chem.*, 46, 1921, 559.
- Osborne, T. B. and Mendel, L. B. The relative value of certain proteins and protein concentrates as supplements to corn gluten. *Ibid.*, 29, 1917, 69.
- Reynolds, E. and Macomber, D. Defective diet as a cause of sterility. A study based on feeding experiments with rats. *J. Amer. Med. Assn.*, 77, 1921, 169.
- Reynolds, E. and Macomber, D. Certain dietary factors in the causation of sterility in rats. *Amer. J. of Obstet. and Gynec.*, 2, 1921.
- Sherman, H. C. and Smith, S. L. The vitamins. *Chem. Cat. Co., N. Y.*, 1922, 273 pp., 8°.

APPENDIX TABLE I

Occurrence of First Oestrus and Ovulation Cycles until Time of Breeding of First Generation of Rats reared on Standard Diet II (Basic Mixture of Casein, Lard, Cornstarch, Salts, Milkfat and Yeast). See APPENDIX TABLE III for Complete Reproductive Performance

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina And First Oestrus	Age In Days at First Oestrus	Length In Days of First Four Cycles	Length In Days of Subsequent Cycles
W 6306	57	0	57	18, 7, 5, 11	9, 7, 5, 4, 5, 5, 4, 4, 5, 6, 4, 5, 5, 6, 4, 4, 4, 6, 7, 8, 12, 6, 12, 14, 4, 6, 4, 5, 4, 4, 7, 4, 6, 4, 6, 5, 5, 4, 6, 4, 6, 4, 4, 4, 4, 5, 4, 9, 7, 7, 4, 6, 20, 2 + died
W 6607	49	1	50	11, 5, 8, 4	6, 5, 4, 4, 4, 4, 4, 4, 4, 4, 8, 4, 4, 4, 5, 5, 4, 4, 13, 4, 6, 4, 5, 5, 5, 5, died
W 6611	67	52	119	19, 6, 9, 48	7, 5, 4, 5, 5, 5, 4, 6, 5, 8, 5, 4, 5
W 6313	58	0	58	29, 11, 9, 9	5, 4, 6, 4, 6, 4, 6, 5, 5, 4, 6, 5, 5, 4, 4, 9, 14, 17, 6, 3 + Lung infection Discarded
B 6317	63	11	74	10, 18, 6, 4	6, 6, 5, 5, 5, 5, 5, 5, 5, 5, 16, 5, 4, 5, 7, 12, 8, 5, 11, 5, 5, 7, 5, 8, 5, 5, 7, 5, 5, 4, 5, 5
BH 6613	55	0	55	22, 7, 5, 11	5, 7, 6, 11, 10, 5, 4, 6, 5, 6, 29, 12, 9, 7, 5, 5, 4, 5, 4, 6, 5, 4
BH 6618	62	10	72	7, 6, 6, 6	10, 5, 7, 15, 12, 20 + Lung infection. Discarded
BH 6625	39	43	82	12, 18, 19, 12	5, 27, 18, 15, 23, 10, 11, 23, 6, 10, 6, 5, 5
W 6327	57	0	57	21, 19, 11, 7	4, 4, 9, 4, 4, 4, 7, 5, 5, 6, 8, 5, 5, 5, 27, 11, 4, 11, 5, 12, 6, 5, 5, 5, 4, 6, 5, 5, 5
W 6551	55	3	58	14, 15, 8, 5	5, 6, 5, 10, 5, 5, 5, 5, 5, 5, 5, 5, 5, 7, 8, 5, 8, 10, 7, 4, 14, 6, 5, 5, 5, 5, 5, 5
GH 6352	43	0	43	7, 5, 5, 22	16, 11, 9, 4, 7, 5, 5, 5, 5, 5, 4, 6, 5, 5, 5, 5, 4, 6, 39 + Lung infection. Discarded

APPENDIX TABLE I—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	No. of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days at First Four Cycles	Length In Days of Subsequent Cycles
W 6554	56	0	56	7, 7, 10, 5	6, 5, 5, 3, 4, 5, 4, 5, 5, 3, 5, 3, 5, 5, 4, 4, 4, 4, 4, 4, 5, 4, 5, 5, 6, 5, 4, 9, 4, 9, 5, 6, 8, 6, 4, 7, 4, 5, 4, 12, 6, 5, 5, 5, 5, 4, 9
W 6356	67	46	113	5, 10, 7, 9	12, 17, 9, 15, 15, 8 + Died
W 6556	72	23	95	7, 15, 6, 9	8, 15, 6, 39 + Died
B 6559	47	52	99	9, 8, 6, 7	6, 4, 13, 6, 19, 4, 18, 20, 5, 7, 8, 6, 4, 5, 3, 5, 5, 5, 5, 10, 4, 4, 5, 4
B 6564	54	0	54	7, 21, 5, 5	6, 5, 6, 5, 6, 6, 5, 5, 6, 8, 5, 6, 5, 70, 5, 6, 6, 3, 7, 4, 4, 4, 4, 4, 5, 5, 10, 5, 5, 5, 5
G 6577	46	6	52	12, 25, 7, 5	11, 5, 5, 5, 5, 6, 4, 5, 5, 4, 6, 8, 5, 7
W 6679	47	7	54	17, 14, 5, 5	22, 6, 7, 34, 14, 12, 5, 5, 7, 5, 3, 5, 5, 5, 5, 5, 5, 7 + Discarded
W 6671	53	0	53	7, 7, 16, 16	74, 15, 13, 5, 14, 5, 10, 12, 9 + Discarded
B 6573	66	78	144	6, 6, 6, 7	5, 8, 15, 7, 5, 5, 4, 6, 7, 9, 6, 6 + Lung infection. Discarded
BH 6978	42	15	57	35, 12, 7, 5	7, 4, 7, 10, 5, 5, 5, 5, 5, 5, 6, 5, 5, 4, 6, 5, 4, 6, 4, 6, 5, 5, 7, 4, 6, 5, 2 + Discarded
W 6940	53	1	54	19, 9, 10, 5	6, 7, 6, 5, 7, 6, 9, 6, 5, 6, 6
W 6905	49	0	49	17, 18, 21, 13	11, 19, 9, 6, 8, 6, 5, 9, 5, 5, 6, 16, 4 + Discarded
W 6744	62	0	62	6, 8, 6, 6	8, 6, 6, 6, 5, 5, 5, 5, 4, 6, 5, 5, 4, 5, 6, 4, 6
B 6769	39	0	39	8, 13, 6, 5	10, 11, 9, 6, 7, 8, 8, 7, 5, 5, 5, 4, 6, 13, 5, 4, 4, 6, 5, 5, 5, 5, 5, 4, 4
W 6754	51	0	51	10, 8, 5, 7	5, 13, 5, 5, 7, 5, 5, 4, 5, 5, 6, 5, 4, 6, 5, 5, 4, 4, 4, 4, 5, 5, 5, 5, 4, 4, 5, 4
W 6751	71	2	73	41, 28, 8, 5	6, 4, 4, 5, 5, 3, 5, 4, 8, 7, 10, 5, 4, 4

APPENDIX TABLE I—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days of First Four Cycles	Length In Days of Subsequent Cycles
BH 6985	49	0	49	21, 20, 13, 15	17, 12, 12, 8, 8, 6, 9, 7, 5, 5, 8, 7, 6, 5, 12, 7, 5, 5, 5, 5, 5, 4, 6, 5, 5, 4, 6, 5, 6, 4, 5, 4, 5, 4, 5, 7, 4, 6, 7, 4, 3 + Discarded.
GH 6982	70	25	95	16, 8, 24, 5	8, 5, 5, 5, 7, 5, 8, 5, 5, 5, 5, 9, 4, 9, 12, 5, 5, 5, 7, 6, 5, 4 + Lung infection. Discarded
G 6975	53	4	57	23, 11, 14, 11	16, 13, 7, 6, 5, 6, 6, 6, 12, 5, 5, 6, 11, 11, 14, 12, 4 + Discarded
B 6955	55	0	55	17, 11, 10, 5	5, 9, 8, 29, 11, 4, 6, 4, 6, 5, 5, 5, 4, 5, 6, 4, 5, 5, 13, 4, 7, 4, 6, 4, 9, 6, 5, 4, 6, 4, 6, 5, 4, 5, 6, 4, 4, 10, 14, 4, 4, 3 + Lung infection. Discarded
W 6844	47	0	47	8, 6, 18, 6	10, 7, 9, 7, 6, 6, 6, 18, 7, 11
G 6934	77	0	77	11, 6, 8, 8	5, 5, 9, 6, 14, 6, 5, 4, 5, 6, 4, 6, 5, 4, 6, 5, 5, 4, 5, 6, 4, 5, 15, 5, 4, 4, 4, 4, 4, 4, 5, 6, 5, 5, 5, 4, 5, 6, 10, 7, 4, 7, 6, 4 + Discarded
BH 6917	54	0	54	12, 13, 5, 10	10, 7, 5, 15, 20, 5, 7 + Lung infection. Discarded
BH 6909	56	1	57	20, 13, 11, 11	7, 13, 15, 12, 7, 4, 6, 5, 6, 5, 6, 5, 5, 5, 5, 5, 5, 6, 6, 6, 9, 5, 5, 6, 6, 4 + Lung infection. Discarded
GH 6847	46	0	46	13, 9, 9, 7	6, 6, 5, 10, 6, 5, 6, 6, 20, 7, 9, 4
B 6805	45	0	45	7, 10, 10, 11	14, 12, 8, 5, 6, 5, 5, 5, 5, 5, 5, 13
W 6808	62	13	75	10, 8, 5, 5	11, 8, 6, 7, 5, 5, 6, 5, 5, 9, 5, 6, 5, 5, 5, 5, 7, 11, 5
B 6812	60	25	85	20, 8, 6, 4	9, 6, 5, 6, 4, 7, 5, 7, 4, 5, 5, 5, 5, 4, 6, 5, 5, 7, 10, 5, 5, 4, 5, 5, 5, 9, 6, 5, 3 + Discarded
BH 6835	49	0	49	5, 10, 17, 13	25, 5, 4, 6, 5, 7, + Died
W 6763	69	27	96	5, 3, 6, 5	4, 19, 5, 11, 7, 4, 6, 5, 5, 6, 20, 5, 5, 5, 5, 14, 3, 13, 6

APPENDIX TABLE 1—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	No. of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days at First Four Cycles	Length In Days of Subsequent Cycles
W 6775	59	37	96	6, 9, 6, 5	5, 5, 4, 6, 6, 5, 6, 6, 4, 5, 5, 5, 5, 5, 5, 5, 4, 6, 4, 5, 4
BH 6864	39	0	39	8, 8, 10, 7	7, 5, 9, 5, 6, 6, 6, 7
GH 6882	67	11	78	13, 8, 10, 6	8, 5, 6, 14, 8, 5, 5, 4, 5
BH 6786	64	41	105	12, 5, 12, 8	4, 10, 4, 5, 5, 6, 5, 5, 5, 7, 5, 5, 4, 6
GH 6900	48	3	51	16, 8, 5, 5	6, 6, 4, 6, 6, 5, 4, 6, 5, 7, 5, 5, 6, 6, 6, 4
B 6895	40	0	40	4, 6, 6, 9	9, 6, 7, 5, 5, 5, 7, 6, 5, 5
W 6943	61	0	61	13, 6, 5, 5	6, 5, 5, 5, 4, 5, 5, 5, 5, 5, 5, 5, 6, 4, 5, 4
W 6945	59	3	62	11, 9, 7, 8	5, 8, 6, 5, 6, 21, 5, 5, 5, 5, 4, 4, 4, 6, 5 + Died
B 6878	58	25	83	27, 41, 5, 6	4, 4, 4
G 7073	53	0	53	18, 6, 6, 4	6, 5, 6, 6, 4, 4, 4, 5, 5, 5, 4, 5, 4, 6, 9, 4, 4, 4, 4, 4
W 7074	53	0	53	9, 11, 5, 6	5, 5, 8, 5, 5, 5, 5, 5, 5, 5, 4, 4, 5, 5, 4, 7, 5, 4, 4, 4, 5
W 7075	52	0	52	14, 12, 16, 21	15, 18, 6, 6, 3, 6, 7, 7
W 7076	46	0	46	12, 10, 7, 7	24, 8, 10, 7, 5, 9, 4, 10, 5, 5, 4, 6
W 7077	72	0	72	9, 5, 15, 12	5, 10, 5, 5, 7, 5, 6, 7, 8, 5, 6, 4
W 7078	47	1	48	10, 11, 5, 9	14, 15, 9, 8, 8, 4, 4, 5, 4, 8, 4, 5, 4, 6, 4
W 7079	41	0	44	12, 13, 7, 5	5, 9, 10, 7, 13, 5, 4, 4, 4, 5, 5, 5, 6, 5, 5, 4, 5, 5
W 7080	48	0	48	6, 6, 8, 5	5, 5, 6, 6, 15, 5, 5, 5, 4, 4, 6, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4
W 7081	60	0	60	11, 7, 4, 4	7, 5, 5, 4, 8, 9, 5, 7, 4, 4, 4, 4, 4, 5, 5, 4, 4, 4, 4, 4, 4, 4



APPENDIX TABLE I—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	No. of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days of First Four Cycles	Length In Days of Subsequent Cycles
W 7082	55	0	55	16, 5, 11, 25	6, 9, 4, 4, 4, 4, 8, 5, 5, 5, 5, 4, 5
GH 7083	68	0	68	6, 7, 6, 5	19, 5, 7, 5, 4, 6, 5, 4, 4, 4, 6, 4, 4, 5, 3, 4, 4, 4, 12
W 7084	61	10	71	13, 5, 15, 13	5, 5, 5, 4, 4, 5, 13, 5, 5, 5, 5, 6
W 7085	68	0	68	9, 5, 6, 5	6, 6, 6, 5, 5, 6, 4, 4, 4, 4, 4, 4, 5, 4, 5, 5, 3, 4, 4, 4
W 7506	55	0	55	8, 9, 10, 8	6, 10
G 7415	48	0	48	8, 5, 5, 5	5, 5, 6, 5, 5, 5, 5, 5
W 7417	47	8	55	9, 12, 5, 7	6, 8, 5, 8
W 7522	43	0	43	8, 6, 5, 5	7, 5, 4, 5, 5, 5, 4
GH 7524	57	1	58	23, 13, 18, 18	
W 7340	46	0	46	7, 4, 15, 4	4, 4, 5, 5, 5, 13
G 7341	61	0	61	13, 7, 9, 5	6, 4, 6
GH 7342	50	0	50	6, 5, 5	Lung infection. Discarded
BH 7345	57	0	57	10, 8, 5, 7	5, 5, 5, 5, 5
W 7346	45	0	45	12, 7, 8, 12	7, 9, 7, 6
W 7347	63	0	63	13, 5, 6, 7	4, 6, 7
B 7352	46	0	46	8, 6, 5, 6	5, 5, 8, 6, 5, 5, 5
W 7353	47	0	47	10, 8, 5, 12	7, 7, 7, 5
BH 7354	43	0	43	9, 7, 7, 7	12, 6, 8, 6, 15
GH 7566	74	0	74	9, 9, 5	
W 7567	34	0	34	6, 7, 4, 6	11, 6, 5, 5, 5
GH 7594	46	0	46	10, 7, 5, 4	4, 6
G 7541	51	0	51	8, 5, 5, 7	5, 5, 5, 5, 6, 5, 4
BH 8320	43	0	43	3, 11, 5, 5	5, 5, 5

APPENDIX TABLE I—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days of First Four Cycles	Length In Days of Subsequent Cycles
BH 8341	68	10	78		
B 9102	43	0	43	8, 11, 6	
B 9103	37	0	37	9, 7, 5, 5	5
BH 8903	38	2	40	11, 7, 7	
W 8911	65	17	82		
BH 8931	45	0	45	5, 7, 5	
W 8937	42	0	42	4, 8, 10	
BH 9069	42	0	42	9, 15, 9	
W 9080	50	1	51		
G 9085	40	7	47	11, 8, 5	
GH 9088	40	5	45	16, 5	
GH 9089	39	2	41	8, 5, 6, 5	5, 5
GH 9090	43	1	44	9, 5, 5, 5	5, 4
GH 9091	56	13	69		
W 9092	38	0	38	5, 5, 5, 4	4, 4
W 9093	39	0	39	9, 6, 7, 6	
B 8896	48	0	48	13, 9	
W 8899	44	0	44	9, 5, 5, 5	4, 4
W 9242	44	0	44	6, 6, 4, 5	
G 9243	46	9	55	8	
W 9048	50	0	50	14, 9	
B 9068	48	0	48	10, 6, 8	
W 9072	40	0	40	6, 5, 5, 5	5, 5
B 9077	46	5	51	9	
G 9081	48	0	48	7, 11, 7	

APPENDIX TABLE I—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	No. of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days of First Four Cycles	Length In Days of Subsequent Cycles
B 9120	45	0	45		
B 9124	42	0	42	11, 8	
BH 9128	46	0	46	8, 5, 5	
GH 9130	44	0	44	10, 7	
W 9173	41	0	41	7, 5, 5, 6	
W 9180	50	0	50	9, 5	
B 9304	40	0	40	8, 5, 4	
W 9298	36	2	38	7, 4, 4	
GH 9066	42	7	49	9 + Discarded	
BH 9136	59	7	66		
W 9311	44	2	46	7, 5	
G 9320	45	2	47	8	
B 9332	48	1	49	8	
B 9290	53	0	53	5	
G 6862	51	0	51	11, 12, 11, 10	11, 11, 5, 5, 6, 14, 8, 6, 5, 4
BH 6988	71	17	88	9, 5, 5, 7	4, 6, 5, 11, 5, 4, 6, 4, 5, 5, 5, 5, 5, 5, 5, 5, 5, 4, 4, 4, 4, 5, 5, 7, 4, 5, 4, 4, 5, 4, 4, 5, 5, 4 + Lung infection. Discarded
B 6890	54	0	54	17, 9, 9, 8	5, 5, 6, 5, 3, 5, 12
W 6822	72	0	72	13, 5, 13, 3	12, 5, 8, 6, 6, 7, 10, 5, 8, 6, 7, 7, 5, 5, 4, 5, 5, 5, 5, 5, 5, 5, 5, 4, 6, 5, 6, 9, 5, 5, 4, 7, 5 + Discarded
W 6901	56	23	79	10, 11, 5, 6	7, 5, 6, 6, 21, 5, 6, 5, 4
GH 6931	47	0	47	5, 12, 17, 12	9, 5, 6, 6, 9, 17, 5, 5, 6, 5, 5
BH 6938	50	0	50	15, 16, 8, 6	7, 6, 8, 8, 15, 6, 7, 4, 4
GH 6852	62	34	96	22, 10, 26, 100	5, 4

APPENDIX TABLE I—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days of First Four Cycles	Length In Days of Subsequent Cycles
W 6856	61	0	61	13, 19, 10, 5	6, 6, 6, 12, 10, 6, 5, 5, 5, 5, 5
W 6757	79	33	112	6, 7, 5, 5	5, 5, 5, 5, 5, 6, 5, 5, 5, 3, 5, 5, 5, 5, 5, 4, 4
BH 6859	55	0	55	9, 10, 8, 7	6, 6, 5, 6, 4, 4, 12, 11, 8, 5, 5, 5, 4, 8, 4
GH 6869	68	10	78	21, 15, 12, 9	
W 7135	53	5	58	9, 7, 5, 6	22, 4, 5, 6
G 7136	74	2	76	17, 14, 9, 6	11
W 7069	73	5	78	10, 31, 7, 8	
B 7070	64	4	68	7, 7, 5, 5	5, 18, 8, 6, 5
BH 7702	34	0	34	8, 5, 5, 5	6, 4, 7, 15, 4, 4, 7
B 7703	43	0	43	6, 4, 6, 5	4, 5, 6, 7, 4, 5, 5, 5
W 7704	58	17	75	6, 9, 12, 5	
GH 7709	43	0	43	8, 6, 11, 25	12
B 7713	64	0	64	13, 17, 10, 4	7, 13, 13, 6, 8, 4, 7, 5, 8 + Diet changed
B 7717	49	0	49	12, 4, 5, 5	4, 5, 5, 4, 14, 5, 5, 6, 4, 5, 6, 4, 5, 5, 6, 4, 6, 5 + Diet changed
W 7719	56	0	56	5, 5, 6, 5	5, 5, 5, 6, 5, 5, 5, 5, 5, 6, 6, 4, 5, 6, 5, 5, 5, 5, 6, 3 + Diet changed
W 7739	41	0	41	7, 7, 7, 6	6, 6, 6, 12, 6, 5, 5, 7, 7, 5, 6, 5, 5, 4, 6, 5, 4, 6, 5 + Diet changed
B 7142	97	13	110	7, 6, 5, 5	6, 4, 6, 5, 5, 5, 4, 6, 5, 5, 4, 5, 6, 4, 6, 5, 5, 4, 6, 5, 4, 4, 4, 4
W 7145	74	0	74	34, 7, 7, 5	5, 5, 5, 5, 6, 4, 5, 5, 5, 5, 5, 5, 5, 4, 6, 6, 5
W 7157	55	0	55	11, 7, 7, 17	6, 5, 5, 5
W 7171	67	35	102	7, 5, 5, 5	5, 6, 5, 5, 5, 5

APPENDIX TABLE I—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days of First Four Cycles	Length In Days of Subsequent Cycles
B 7186	49	0	49	8, 9, 9, 19	9, 5, 6, 4, 4, 5, 5, 4, 6, 5, 4, 4, 5
B 7196	103	0	103	9, 17, 8, 5	5, 6, 5, 6, 5, 5, 12, 6, 6, 5, 5, 6, 5, 4, 5, 6, 4, 6, 6, 6, 6
BH 7743	61	0	61	10, 5, 10, 10	13, 6, 11, 9, 6, 8, 6, 4, 8, 7, 5, 5 Diet changed
W 7745	52	6	58	5, 6, 7, 4	6, 7, 5, 6, 11, 11, 6, 13, 6
G 7748	51	0	51	4, 6, 5, 5	4, 4, 12, 7, 10, 4, 7, 4, 6, 4, 5, 5, 5, 5, 5, 6, 4, 5, 5, Diet changed
W 7692	42	0	42	8, 5, 4, 6	5, 5, 4, 5, 4, 5, 5, 6
BH 7697	51	0	51	8, 7, 4, 5	12, 5, 5, 4
BH 7602	66	12	78	16, 12, 7	
B 7603	43	0	43	8, 12, 6, 8	14, 8, 5
W 7609	62	0	62	6, 5, 8, 5	7, 6, 6
G 7598	42	11	53	10, 5, 5, 21	11
G 7599	66	0	66	9, 14, 9, 9	16
B 7948	38	0	38	7, 6, 12, 5	6, 5, 5, 5, 5, 5, 4
B 7949	39	0	39	7, 6, 8, 6	6, 8, 5, 6, 6, 9, 6, 6, 4, 6, 5, 5, 6, 5, 4, 5, 4, 6, 3, 4, 4, 5, 5 + Lung infection. Discarded
W 7952	43	0	43	6, 8, 8, 10	8, 13, 7, 4, 9, 5, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 6, 4, 4, 4
B 7958	55	11	66	11, 7, 11, 18	
B 7959	52	12	64	16, 13, 10	
W 7964	47	0	47	10, 29, 14	
W 7965	50	0	50	12, 29, 36, 11	7
W 7323	43	0	43	13, 7, 5, 5	5, 4, 6, 5, 6, 4, 6, 4, 6, 5, 12, 4
W 7438	44	0	44	5, 5, 5, 4	6, 4, 4, 4, 4, 4, 4, 4, 4, 3 + Discarded

APPENDIX TABLE I—*Continued*

Designation of Rat	Age In Days at Rupture of Vaginal Closing Membrane	No. of Days Between Opening of Vagina and First Oestrus	Age In Days at First Oestrus	Length In Days of First Four Cycles	Length In Days of Subsequent Cycles
W 7542	47	0	47	9, 8, 5, 6	10, 5, 9, 5, 6, 6, 5, 6
G 7453	66	0	66	10, 10, 6, 7	5, 5, 5, 5, 5, 4, 7, 6, 7, 5, 5, 5, 4, 6, 6, 5, 5, 6, 5, 5, 5, 11, 7, 4, 6, 4, 7, 6, 6, 20, 6, 8 + Discarded
W 7459	38	0	38	5, 10, 8, 6	6, 4, 5, 6, 6, 6, 6, 5, 5, 9, 13, 2 + Discarded
B 7464	70	0	70	10, 17, 12, 11	11, 15, 12, 7
B 7513	55	1	56	14, 6, 6, 15	7, 4, 5
G 7514	47	9	56	15, 9, 5, 6	8, 6, 5
W 8354	56	0	56	8, 9, 6, 8	7, 8, 4, 4, 6, 4, 6, 4, 4, 5, 5
B 8357	43	0	43	10, 8, 7, 6	7, 8, 7, 6, 5, 5, 5, 4, 6, 5, 5
B 8363	63	3	66	13, 11, 8, 5	5, 6, 5, 5, 4, 6, 5
GH 8432	40	0	40	8, 7, 8, 7	6, 5, 5, 7, 5, 5, 6, 5, 5, 6, 6, 6, 7, 10
B 8410	35	0	35	5, 7, 5, 5	6, 5, 6, 7, 6, 5, 6, 4, 7, 6, 5, 4, 5, 5, 5, 5, 6, 5
W 8438	38	3	41	5, 4, 5, 4	4, 4, 5, 4, 5, 6, 5, 4, 6, 5, 6, 5, 5, 6, 5, 7, 5, 5
BH 7071	61	0	61	12, 16, 23, 9	9, 5, 4, 5, 5, 5, 6, 4, 5, 5, 6, 5
B 7072	113	13	126	8, 5, 5, 5	4, 9, 5, 5, 7, 4, 4
B 9107	52	0	52	4, 5	
B 9114	63	8	71		
BH 9126	50	0	50	5, 4, 5	
B 9178	62	0	62		
W 9285	47	0	47	7	
BH 9142	53	15	68		
G 9186	75	8	83		



APPENDIX TABLE II

Occurrence of first oestrus and ovulation cycles until time of breeding of second generation of rats reared on Standard Diet II (basic mixture of casein, lard, cornstarch, salts, milkfat, and yeast). See APPENDIX TABLE IV for complete reproductive performance.

Litter of	Designation of Female	Age in Days at Rupture of Vaginal Closing Membrane	Number of Days between Opening of Vagina and First Oestrus	Age in Days of First Oestrus	Length in Days of First Four Cycles				Length in Days of Subsequent Cycles
					6	5	5	5	
Litter of B 6864:	W 7724	39	5	44	6	5	5	5	5, 5, 5, 5, 5, 4, 6, 6, 15.
	BH 7726	38	0	38	7	5	7	5	5, 5, 5, 5, 5, 5, 5, 5.
Litter of W 6327:	BH 7727	35	0	35	5	6	7	5	5, 5, 5, 5, 5, 5, 5, 5.
Litter of W 6611:	G 7734	49	0	49	10	32	10	6	14, 10.
	B 7737	60	10	70	7	6	5	8	10, 5, 7, 4, 6.
Litter of B 6310:	G 7738	68	0	68	8	6	5	5	6, 6, 5, 5, 5, 5.
	W 7862	40	0	40	5	6	5	6	6, 6, 5, 5, 5, 4, 6, 4, 6, 5, 4.
Litter of GH 6951:	B 7863	35	0	35	5	5	5	5	7, 6, 6, 6, 4, 6, 5, 5, 4, 5, 5, 6.
Litter of W 6943:	W 7870	67	0	67	7	5	6	7	5, 4, 6, 5, 5.
	W 7871	41	0	41	13	7	5	7	4, 5, 4, 6, 5, 5, 5, 4, 5.
	W 7872	39	0	39	8	10	7	4	6, 4, 4, 4, 4, 4, 4, 4.
	W 7873	46	0	46	13	8	15	5	4, 4, 6, 4, 5, 6.
Litter of GH 6869:	W 7889	62	0	62	11	6	6	5	7, 5, 5, 6, 5, 5.
Litter of B 6769:	G 7940	57	0	57	8	6	4	7	5, 5, 8.
	W 7941	48	0	48	10	18	6	7	10, 18, 19, 10, 9, 11.
Litter of W 6901:	W 8096	68	0	175	5 + prolonged oestrified	5	5	7	Killed.
Litter of W 6478:	W 8097	60	0	60	8	5	4	5	5, 6, 5.
	W 8098	51	0	51	14	10	9	8	5, 5, 5.
	W 8099	52	0	52	9	7	6	5	5, 5, 5 + Killed.
	W 8100	61	2	63	10	7	5	5	5, 11, 5, 6, 8, 5, 5, 5, 5, 15, 5.
	W 8101	51	0	51	13	6	6	5	5, 6, 4, 6.

Litter of G 7139:	B 8113	55	0	55	9	7	6	5	6, 5, 5.
Litter of B 5655:	G 8114	51	0	54	4	5	5	5	4, 4, 1, 4, 4, 4, 1, 4, 1, 4, 1, 4, 1.
	B 8117	61	0	61	5	5	5	5	Lung infection. Discarded.
	B 8118	59	0	59	5	6	5	5	5, 4, 5, 2 + Lung infection. Discarded.
	BH 8119	54	0	54	6	6	5	4	6, 5, 5, 7, 5, 4, 3. Lung infection. Discarded.
	BH 8120	53	1	51	7	4	6	4	4, 6, 4, 4, 4, 5, 5, 5, 6, 4, 5, 4, 6, 8.
Litter of W 6856:	BH 8139	50	0	50	6	6	5	4	5, 5, 5, 5, 6.
	BH 8140	73	0	73	5	9	6	7	16.
	BH 8141	53	0	53	7	10	6	5	Lung infection. Discarded.
Litter of BH 6938:	BH 8142	50	0	50	6	7	5	6	9, 6, 4, 6, 8, 6, 5, 6, 6, 5.
	BH 8151	45	0	45	10	5	7	5	5, 2 + Lung infection. Discarded.
Litter of G 6565:	W 8152	47	0	47	20	7	14	22	14, 6, 7, 7, 5, 6.
Litter of W 7506:	G 8204	61	0	61	15	6	5	4	5, 9, 6, 4, 15, 4.
	G 8278	52	0	52	4	7	4	5	4, 4, 4.
	B 8279	51	0	51	7	10	7	8	
	W 8280	61	0	61	9	7	4	4	
Litter of W 7076:	G 8271	56	0	56	5	6	6	4	4.
	B 8286	40	1	41	6	5	5	6	5, 4, 3 + Lung infection. Discarded.
Litter of B 7543:	W 8327	57	9	66	6	8	6	6	
	B 8328	49	0	49	7	7	5	6	
	W 8345	39	0	39	12	5	5	5	
Litter of W 7075:	W 8346	42	0	42	8	5	9	6	
	W 8347	39	0	39	5	7	6	5	5, 5, 6.

## APPENDIX TABLE III.

Reproductive Performance of First Generation of Rats Reared on Standard Diet II (Basic Mixture of Casein, Lard, Cornstarch, Salts, Milkfat and Yeast.)  
See APPENDIX TABLE I for occurrence of oestrus and ovulation cycles.

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental sign (r. b. c.)	Litter	NUMBER OF YOUNG		AVERAGE WGT. OF YOUNG (in gms.)		WEIGHT OF MOTHER (in gms.)		ANTENATAL RECORD						Averages (in gms.) of foetuses	Notes
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora lutea		Living foetuses		Resorptions			
												R	L	R	L	R	L		
W 7079	6 mos.	B 6145	•	•	•	5	1	4.8	20.0	240	272							2 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.	
W 7080	6 mos.	W 52	•	0	•	4	0	5.5		258								Litter lived one day only. Autopsied 19th day of gestation.	
W 7081	6 mos.	B 7175 BH 7305	•	•	•	•	•	•	•	•	•							Autopsied 19th day of gestation. Litter lived 4 days only.	
W 7082	6 mos.	B 7307	•	•	•	4	0	4.7		270								Autopsied 19th day of gestation. Litter lived 1 day only.	
GH 7083	6 mos.	BH 50 W 26	•	•	•	4	0	3.5		286								Autopsied 19th day of gestation. 4 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.	
W 7084	6 mos.	W 7312 B 68	•	•	•	(1 dead)												Autopsied 19th day of gestation.	
W 7085	6 mos.	W 5400	•	•	•	6	4	6.0	23.5	267	295							Autopsied 19th day of gestation.	
W 7506	4 mos.	W 00 B 45 GH 00	•	•	•	7	6	6.0	22.1	240	248							Autopsied 19th day of gestation. 4 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.	
G 7415	4 mos.	BH 00 W 31	•	•	•	4	0			267								Autopsied 19th day of gestation. 4 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.	
B 6317	10 mos.	B 87	•	•	•	5	1	4.8	not weighed	328	415							Autopsied 20th day of gestation.	
W 6327	10 mos.	00 B 7307 W 04	•	•	•	2	2	6.5	40.5	306	300							Autopsied 19th day of gestation. 4 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.	
W 6351	8 mos.	BH 76 B 6251	•	•	•	3	0	9.0		296								Autopsied 19th day of gestation. 4 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.	
B 6550	9 mos.	W 00 7306	•	•	•	3	1	7.1	30.0	284	270							Autopsied 19th day of gestation.	

W 6564	10 mos.	W 00 G 6851	.	.	.	.	3	0	0	0	258	288	5	3	0	1	1	1	1.8 Autopsied 19th day of gestation. Litter lived 2 days only. Died 7 days after next mating.
W 6614	10 mos.	BH 00 W 40 G 7180 B 40 B 5719	.	.	.	.	6	3	5	3	230		2	7	2	6	0	0	3.1 Autopsied 21st day of gestation. Marked variation in size of foetuses.
W 6751	8 mos.	GH 10 B 08 BH 00 W 14 W 46 W 20 GH 70	.	.	.	.	3	0	5	6	324		5	6	0	0	0	6	Autopsied 19th day of gestation
W 6754	7 mos.		.	.	.	.	0	0					5	3	1	0	4	2	1.9 Autopsied 19th day of gestation.
B 6769	7 mos.		.	.	.	.	6	6	0	2	246	250	4	1	3	3	1	1	Autopsied 19th day of gestation. 1 more matings without placental sign.
W 6775	7 mos.		.	.	.	.	0												Autopsied 2 days after mating. Infected.
BH 6786	7 mos.	W 91	.	.	.	.	0												1 more matings without placental sign. Autopsied 2 days after mating. Infected.
W 6808	7 mos.	W 00 W 31 W 512 B 51	.	.	.	.	0						1	0	0	0	0	3	Autopsied 19th day of gestation. Autopsied 19th day of gestation.
W 6822	10 mos.	W 512	.	.	.	.	0				300	309	2	1	0	0	2	4	Autopsied 19th day of gestation.
W 6841	6 mos.	BH 51	.	.	.	.	2	2	6	5	10	5	3	5	0	3	0	2	2.2 Autopsied 19th day of gestation.
GH 6847	6 mos.	W 01 W 11	.	.	.	.	0												4 more matings without placental sign. Autopsied 2 days after mating. Infected.
W 6856	6 mos.	BH 27 W 20 B 7182 W 40	.	.	.	.	8	5	5	5	280	313	5	4	0	0	0	4	Autopsied 19th day of gestation. Sudden loss in weight occurred at close of gestation period. 4 more matings without placental sign. Autopsied 2 days after mating. Infected.
G 6862	5.5 mos.		.	.	.	.	?	no litter found											
BH 6864	4 mos.	BH 7301	.	.	.	.	5	5	not weighed	not weighed	208	240	1	6	0	5	0	0	3.9 Autopsied 21st day of gestation.
		W 40 W 40 GH 65 BH 7304 BH 00 B 473	.	.	.	.	6	3	5	8	251	300							Became sickly. Discarded.
B 6878	5 mos.		.	.	.	.	8	0	4	0	252								
		W 7306 G 7180 W 01 W 26	.	.	.	.	2	2	6	0	333		10	2	5	0	1	0	1.7 Autopsied 10th day of gestation
GH 6882	6 mos.		.	.	.	.	0						8	4	4	2	2	2	2.2 Autopsied 19th day of gestation.
B 6890	4.5 mos.	B 69 GH 54 W 14	.	.	.	.	0												5 more matings without placental sign. Autopsied 2 days after mating. Infected.
B 6895	4.5 mos.	GH 70	.	.	.	.	7	0	4	0	222								Autopsied 2 days after next mating. Normal.

APPENDIX TABLE III.—Continued

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Litter	NUMBER OF YOUNG		AVERAGE WGT. OF YOUNG (in gms.)		WEIGHT OF MOTHER (in gms.)		AUTOPSY RECORD				Notes
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora lutea	Living foetuses	Resorptions	Average Weight of Foetuses in Gravid	
G 6900	6 mos.	W 00 B 07	• •	• •	0 0											3 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection.
W 6901	6 mos.	BH 49 W 94 B 23 BH 66 BH 66 G 7180 GH 70	• • • • • • •	• 0 0 0 0 •	•	4	4	5.5	29.7	290	280					
W 7135	6 mos.				0 0											
G 7136	6 mos.	G 8851 W 26 B 6145	• • •	• • •	•	8	4	5.8	35.2	264	284					Autopsied 19th day of gestation. 3 more matings without placental sign. Autopsied 2 days after mating. Infected.
W 7069	4 mos.				•	2	0	4.0		252		7	4	0	4	2
B 7070	4 mos.	G 67 W 66 W 36	• • •	• • •	•	7	0	5.6		294		8	3	0	2	3
W 7323	4.5 mos.	BH 7304 BH 36 BH 45 BH 32	• • • •	• • • •	•											Autopsied 19th day of gestation. 4 more matings without placental sign. Autopsied 2 days after mating. Infected.
W 7542	4.5 mos.				•	1 (dead)	0	4.0		241		4	7	3	0	1
B 7164	6 mos.	W 04 BH 7305 W 31 W 35	• • • •	• • • •	•											Autopsied 16th day of gestation. Autopsied 19th day of gestation. Autopsied 19th day of gestation.
B 7950	3 mos.	W 35 W 33 W 45	• • •	• • •	•							7	6	0	0	7
W 7952	6 mos.	W 45 W 5400 B 88	• • •	• • •	•							2	7	0	0	6
B 7955	4 mos.	W 04	•	•	•											Autopsied 19th day of gestation. Discarded because of abnormal cycles. Diet changed.
W 7522	4 mos.	BH 5719	•	•	•	1	0	5.0		273		5	5	0	0	4
W 7340	4 mos.	BH 27	•	•	•	6 (3 dead)	0	5.3		228						Autopsied 11th day of gestation. Diet changed. 4 more matings without placental sign. Autopsied 2 days after mating. Possibility of infection. Litter lived one day only.

[illegible]







APPENDIX TABLE IV

Reproductive performance of second generation of rats reared on Standard Diet II (basic mixture of casein, lard, cornstarch, salts, milkfat and yeast.) See APPENDIX TABLE II for occurrence of oestrus and ovulation cycles.

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r, b, c,)	Litter	Notes
W 7724	5 mos.	W 6300	"	"	0	Diet changed.
BH 7726	5 mos.	W 26	"	"	0	Diet changed.
BH 7727	5 mos.	W 6500	"	"	0	Diet changed.
G 7734	4 mos.	W 00	"	"	0	Diet changed.
B 7737	4 mos.	B 45	"	"	0	Diet changed.
G 7738	4 mos.	W 7312	"	"	0	Diet changed.
W 7862	4 mos.	BH 81	"	"	0	Diet changed.
B 7863	4 mos.	W 55	"	"	0	Diet changed.
W 7870	4 mos.	W 39	"	"	0	Diet changed.
W 7871	4 mos.	B 40	"	"	0	Diet changed.
W 7872	4 mos.	BH 46	"	"	0	Diet changed.
W 7873	4 mos.	B 48	"	"	0	Diet changed.
GH 7889	4 mos.	W 7312	"	"	0	Diet changed.
G 7940	3 mos.	GH 75	"	"	0	Diet changed.
W 7941	5 mos.	GH 35	"	"	0	
		BH 49	"	"	0	
		W 39	"	0		Lung infection; killed 11th day after mating; not pregnant.
W 8097	3.5 mos.	W 20	"	"	0	Diet changed.
W 8098	3.5 mos.	W 55	"	"	0	Diet changed.
W 8101	3.5 mos.	BH 75	"	"	0	Diet changed.
B 8113	3 mos.	W 03	"	"	0	Diet changed.
G 8114	5 mos.	W 8705	"	"	0	
		W 66	"	0		
		W 55	"	"	0	Diet changed.
BH 8140	3 mos.	B 00	"	0		
		W 39	"	"	0	
		W 39	"	"	0	
		W 66	"	0		
		W 5400	"	"	0	3 more matings without placental sign. Killed 2 days after mating. Infected.
BH 8142	5 mos.	W 8709	"	"	0	
		B 00	"	"	0	
		W 39	"	"	0	
		W 36	"	0		Diet changed.
W 8139	3.5 mos.	G 13	"	"	0	Diet changed.
W 8152	5 mos.	B 66	"	"	0	
		W 73	"	"	0	Diet changed.
BH 8120	5 mos.	W 7312	"	"	0	Diet changed.
G 8271	3.5 mos.	W 02	"	"	0	
		W 8712	"	"	0	
		W 06	"	"	0	Diet changed.
G 8278	3 mos.	W 37	"	"	0	
		W 5400	"	"	0	
		B 8704	"	"	0	Diet changed.
B 8279	3 mos.	W 39	"	"	0	
		W 45	"	"	0	Diet changed.
W 8280	3 mos.	BH 46	"	"	0	
		B 68	"	"	0	
		W 55	"	"	0	Diet changed.
W 8327	3 mos.	W 66	"	"	0	
		B 6706	"	0		
		B 32	"	"	0	
		B 49	"	"	0	Diet changed.
B 8328	3 mos.	W 52	"	"	0	
		W 82	"	"	0	Died 22nd day of gestation.
W 8345	2.5 mos.	B 45	"	"	0	
		G 10	"	"	0	
		BH 49	"	"	0	Diet changed.
W 8346	2.5 mos.	G 10	"	"	0	
		B 22	"	"	0	
		B 90	"	"	0	Diet changed.
B 8347	2.5 mos.	W 82	"	"	0	
		W 39	"	"	0	
		W 00	"	"	0	Diet changed.

Totals: 34 rats.  
63 positive matings.  
57 findings of placental sign.  
0 litters.

APPENDIX TABLE V.

Showing reproductive performance of rats reared on the basic ration (casein 18, cornstarch 54, lard 15, salts 4, milkfat 9, yeast 6 gm., daily), and, after sterility was proven, transferred to the same ration modified either by the addition, in each instance, of a single natural foodstuff or by an alteration in the proportions of the constituents. (Text tables V to XVII give summaries of this table. The occurrence of first oestrus and involution performance of these rats is given in Appendix tables I and II.)

Designation of Female	History on the Basic Diet				Single Added Foodstuff or Modification of Basic Diet	Time of Modification of Diet with Reference to Next Gestation	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.)	Litter	Number of Young		Average Weight of Young (in Grams)		Weight of Mother (in Grams)		Notes
	Age at Breeding	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.)	Number of Litters							At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	
BH 8142	5 mos.	4	2	0	Lettuce—40 gms. fresh leaves daily (See text table V)	3 days before mating	W 10 442	• •	• •	• •	5 (1 dead) 8	4 5	6.7 5.1	38.2 29.2	261 280	262 285	Second generation on the basic ration. Lettuce added at 7 months.
W 7871	4 mos.	1	1	0		1 day before mating	W 65 B 66 B 60	• •	• •	• •	7 12 (1 dead)	6 6	6.3 6.4	46.3 38.3	252 262	248 252	Second generation on the basic ration. Lettuce added at 4.5 months.
W 8152	5 mos.	2	2	0		2 days after mating	B 23 464 541	• •	• •	• •	1 (1 dead) 5	2 5	4.2 6.0	20.0 27.0	226 290	278 290	Second generation on the basic ration. Lettuce added at 7 months.
W 8327	3 mos.	1	3	0		4 days before mating	W 6905 553	• •	• •	• •	1 (dead) 5 (1 dead)	4 4	4.0 6.0	36.0 36.0	201 219	236	Second generation on the basic ration. Lettuce added at 5.5 months.
W 7873	4 mos.	1	1	0		1 day before mating	W 94 W 66 B 704 W 55	• •	• •	• •	• •	• •	• •	• •	• •	• •	Second generation on the basic ration. Lettuce added at 4.5 months.
W 8239	5.5 mos.	1	1	0		4 days before mating	BH 6575 381	• •	• •	• •	3 (1 dead) 2	2 2	7.7 7.0	49.0 46.0	300 368	342 395	First generation on the basic ration. Fed 1 gram yeast daily. Lettuce added at 6 months.
W 8174	5.5 mos.	1	1	0		1 days before mating	B 7851	• •	• •	• •	8	6	6.0	27.0	292	260	First generation on the basic ration. Fed 1 gram yeast daily. Lettuce added at 5 months.
BH 8120	5 mos.	1	1	0	Raw Beef Mince F—10 gms. daily (See text table VI)	6 days before mating	W 93 W 39 W 7093	• •	• •	• •	• •	• •	• •	• •	• •	• •	Second generation on the basic ration. Raw beef muscle added at 6 months.
B 7737	3 mos.	1	1	0		1 day before mating	BH 81 W 5400 W 66	• •	• •	• •	3	1	6.0	17.0	320	355	17 gram drop in weight. No litter found. 6 gram drop in weight. 14 gram drop in weight. 3 more matings without placental sign. Autopsied. Possibility of infection. Second generation on the basic ration. Raw beef muscle added at 4.5 months.

APPENDIX TABLE V.—Continued

Designation of Female	History on the Basic Diet				Single Added Foodstuff or Other Modification of Basic Diet	Time of Modification of Diet with Reference to Next Gestation	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. o.) Found	Litter	Number of Young		Average Weight of Young (in Grams)			Notes	
	Age at Breeding	Sperm or Copulatory Plug Found	Placental Sign (r. b. o.) Found	Number of Litters							At Birth	At Weaning	At Weaning	At Littering	At Weaning		
W 8172	6 mos.	2	2	0		6 days before mating	G 7818 384	• •	• •	3 (1 dead) 3	0 1	5.0 5.7	• • 29.0	260 326	376	Young lived 7 days only. First generation on the basic ration. Fed 4 gram yeast daily. Raw beef muscle added at 8 months.	
BH 8252	5 mos.	1	1	0		day of mating	W 6909 552	• •	• •	40 11	6 5	6.0 5.5	37.8 41.0	300 345	365	First generation on the basic ration. Fed 4 gram yeast daily. Raw beef muscle added at 7 months.	
W 8438	5 mos.	1	1	0	50% WHOLE WHEAT (See text table VII)	4 days before mating	BH 7632	•	•	6	5	5.7	38.0	232	246	First generation on the basic ration. Whole wheat added at 5.5 months.	
BH 8432	5 mos.	1	1	0		5 days before mating	W 7743 453	• •	•	•	•	•	•	•	324	First generation on the basic ration. Whole wheat added at 5.5 months.	
G 8331	5.5 mos.	1	1	0		3 days before mating	W 7256	•	•	•	3	3	6.7	40.0	255	252	Second generation on the basic ration. Whole wheat added at 6 months.
W 8719	4 mos.	1	1	0	1/4 WHEAT EMBRYO (See text table VIII)	1 day before mating	472	•	•	•	7	6	5.7	41.4	240	248	First generation on the basic ration. Wheat embryo added at 5 months.
W 7724	4 mos.	1	1	0	FRESH MILK at lib. (See text table IX)	16 days before mating	B 7523 B 23 W 00	• • • •	• • • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	Second generation on the basic ration. Milk added at 5 months.
BH 7727	1 mos.	1	1	0		8 days before mating	W 11 W 93	• • • •	• • • •	• • • •	1 (dead) •	• •	3.0 •	• •	264	•	Second generation on the basic ration. Milk added at 5 months.
W 7962	3.5 mos.	2	2	0		2 days before mating	B 66	•	•	•	•	•	•	•	•	•	First generation on the basic ration. Milk added at 6 months.
B 7959	3 mos.	2	2	0	1/4 WHOLE MILK POWDER (See text table X)	5 days after mating	W 00 W 93 W 00	• • • • • •	• • • • • •	• • 5	• • 5	• • 5.6	• 38.2	• 230	292	•	First generation on the basic ration. Whole milk powder added at 4 months.
W 7862	4 mos.	1	1	0		4 days before mating	B 00 B 00	• • • •	• • • •	• •	• •	• •	• •	• •	• •	• •	Second generation on the basic ration. Whole milk powder added at 4 months.
BH 7889	4 mos.	1	1	0		1 day after mating	W 5400 B 00 W 5 W 35	• • • • • • • •	• • • • • • • •	• • • •	3 •	• • • •	• • • •	• • • •	294	•	Young lived 6 days. Second generation on the basic ration. Whole milk powder added at 5 months.
G 7731	4 mos.	1	1	0	1/4 WHOLE MILK POWDER (See text table XI)	3 days before mating	B 45	•	•	•	•	•	•	•	•	•	Second generation on the basic ration. Whole milk powder added at 5 months.

APPENDIX TABLE V.—Continued

G	3.5 mos.	3	3	0	½ POWDER (See text table X1)		6 days after mating	W 60 W 7473 W 6936 516	*	0	.	.	.	Second generation on the basic ration. Whole milk powder added at 5 months.
B 8279	3 mos.	2	2	0			7 days after mating	W 493 W 6439	.	0	.	.	.	Second generation on the basic ration. Whole milk powder added at 5.5 months.
W 8280	3 mos.	3	3	0			11 days after mating	B 468 B 7460 G 7785 392	.	0	.	.	.	Second generation on the basic ration. Whole milk powder added at 5.5 months.
B 8999	3 mos.	1	1	0	24% Milk Fat (See footnote text table X)	Day of mating		556	.	.	8	5	4.5 25.4 210	205 Second generation on the basic ration modified so as to contain 25% whole milk powder. 21% milkfat added at 3 months.
G 9000	3 mos.	1	1	0		3 days before mating		563	.	.	6	6	0 28.3 212	220 Second generation on the basic ration modifi- ed so as to contain 25% whole milk powder. 21% milkfat added at 3 months.
G 9001	3 mos.	1	1	0		4 days after mating		463	.	.	7 (4 dead)	0	6.0 . . .	185 1 young partly eaten. 3 young lived 4 days. Second generation on the basic ration modified so as to contain 25% whole milk powder. 21% milkfat added at 3 months.
B 8410	5 mos.	1	1	0	ORANGE JUICE— 10 c. daily (See text table X1)	2 days before mating		W 7011 W 380	.	0	.	.	.	First generation on the basic ration Orange juice added at 6 months.
BH 8714	4 mos.	1	1	0		1 day before mating		581 562	.	0	.	.	.	First generation on the basic ration. Orange juice added at 5 months.
W 8229	6 mos	1	1	0		11 days before mating		555	.	.	5	5	4.6 29.6 188	185 Second generation on the basic ration. Fed orange juice until 51 days old, when first craunts occurred, and orange juice was discontinued. Orange juice added at 6.5 months.
B 8752	4 mos.	1	1	0		1 day before mating		BH 8552	.	0	.	.	.	First generation on the basic ration. Reared on Diet 38. Transferred to Standard Diet 11 at 3 months. Orange juice added at 5 months.



APPENDIX TABLE V. (Continued)

Designation of Female	History on the Basic Diet				Time of Modification of Diet with Reference to Next Gestation	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Number of Young		Average Weight of Young (in Grams)		Weight of Mother (in Grams)		Notes
	Age at Breeding	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Litters					At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	
W 8346	2.5 mos.	3	3	0	9% Codliver Oil substituted for 9% milkfat (See text table XIII)	BH 75 BH 7632 BH 389 469	• • • •	• • • •							Second generation on the basic ration. Codliver oil added at 5 months.
R 8347	2.5 mos.	3	3	0	Day of mating	W 48 W 7845	• •	• •							Second generation on the basic ration. Codliver oil added at 5 months.
B 8357	1.5 mos.	1	1	0	1 day before mating	W 7102 W 7093 458	• • •	• • •							First generation on the basic ration. Codliver oil added at 5 months.
G 7940	3.5 mos	1	1	0	2 days before mating	G 10 B 45 B 100 W 7256	• • • •	• • • •							Second generation on the basic ration. Codliver oil added at 4 months.
W 8097	3.5 mos.	1	1	0	2 days before mating	W 66 B 66 G 7180	• • •	• • •							Killed on 12th day of gestation; right ovary 3 corpora, left ovary 4 corpora; right uterine horn 1 resorption, no foetuses, left uterine horn no resorptions, no foetuses.
W 8217	5 mos.	1	1	1	7 days after mating	G 7785 469	• •	• •							Second generation on the basic ration. Codliver oil added at 4 months.
W 8101	3.5 mos.	1	1	0	4 days before mating	W 93 W 5400 BH 49	• • •	• • •							First generation on the basic ration, modified to contain 24% milkfat and fed 4 grams yeast daily. (Litter of 2, not suckled.) Codliver oil added at 7 months.
B 7863	4 mos.	1	1	0	2 days before mating	W 66 W 52 W 02 W 20	• • • •	• • • •							Second generation on the basic ration. Yeast and casein added at 4 months.
W 8129	3.5 mos.	1	1	0	4 days before mating	B 6678 W 39 W 55 BH 32	• • • •	• • • •							Second generation on the basic ration. Lactalbumen in place of casein added at 5 months.
															Second generation on the basic ration. 50% casein started at 4 months.

APPENDIX TABLE VI

Showing the occurrence of first oestrus and ovulation cycles (up to the time of breeding) of rats reared on the Basic Diet (casein 18, cornstarch 54, lard 15, salts 4, milkfat 9, yeast .6 gm. daily) to which had been added in each instance a single natural foodstuff or in which the proportions of the constituents had been altered. (The reproductive history is given in APPENDIX TABLE VII.)

Single Added Foodstuff or Other Modification of the Basic Diet	Designa- tion of Rat	Age in Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina and First Oestrus	Age in Days at First Oestrus	Length in Days of First Four Cycles	Length in Days of Subsequent Cycles
LETTUCE—40 gms. fresh leaves daily (See text table XVIII)	B 8859	43	0	43	5, 7, 4, 4,	4, 4, 4.
	B 8862	43	0	43	8, 5, 5, 4,	4, 5.
	B 8863	54	2	62	5, 4, 4.	
	BH 8864	45	0	45	9, 4, 4, 4,	4, 4, 4.
	W 9059	48	0	48	6, 6, 6.	
	W 9062	53	0	53	11.	
	W 9063	47	0	47	12, 4, 4, 5.	
	W 9061	43	4	47	6, 5, 6.	
RAW BEEF MUSCLE 5 gms. daily (See text table XIX)	GH 8905	41	0	41	7, 5, 5, 4,	4.
	W 8909	54	0	54	5, 6.	
	BH 8925	41	1	42	7, 6, 5, 5,	5.
50% WHOLE WHEAT (See text table XX)	BH 8996	50	2	52	11, 9.	
	BH 8997	46	4	50	7, 5.	
FRESH WHOLE MILK 5 cc. daily (See text table XXI)	B 8890	57	0	57	7, 6.	
	G 8891	54	0	54	4, 5, 5.	
	B 8902	53	0	53	11.	
FRESH WHOLE MILK 10 cc. daily (See text table XXII)	G 8892	41	0	41	8, 11, 7.	
	G 8893	40	0	40	4, 7, 6, 6,	5.
	GH 8904	46	0	46	10, 3, 7.	
1/3 SKIM MILK POWDER (See text table XXIII)	W 9074	36	0	36	7, 6, 5, 5,	5, 4.
	W 9094	45	0	45	9, 7.	
	W 9095	45	0	45	5, 5, 5.	
	W 9070	46	0	46	4, 5, 5, 5,	4.
ORANGE JUICE 8 cc. daily (See text table XXIV)	B 8894	47	0	47	8, 7, 5.	
	W 8898	50	9	59	6.	
	B 8901	40	0	40	10, 9, 5.	
24% MILKFAT AND .4 GRAM YEAST DAILY (See text table XXV)	W 8164	40	1	41	12, 5, 6, 4,	7, 5, 5, 6, 5.
	G 8171	46	0	46	9, 7, 6, 5,	7, 6, 5, 5, 5, 5.
	W 8187	57	8	65	14, 11, 6, 5.	
	W 8214	48	13	61	9, 5, 8, 7,	6, 7, 5, 6, 5, 4, 7. 6, 5, 4, 6, 5, 4, 6

APPENDIX TABLE VI—Continued

Single Added Foodstuff or Other Modification of the Basic Diet	Designa- tion of Rat	Age in Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina and First Oestrus	Age in Days at First Oestrus	Length in Days of First Four Cycles	Length in Days of Subsequent Cycles
24% MILKFAT AND .4 GRAM YEAST DAILY (See text table XXV)	W 8217	46	0	46	9, 17, 17, 20,	5, 6, 7, 7, 6, 5, 5, 6, 5, 4.
	W 8230	55	8	63	5, 11, 6, 5,	5, 6, 6, 6, 5, 5, 6, 5, 4, 5, 6, 5, 4, 5.
	W 8240	40	0	40	5, 10, 7, 5,	5, 5, 5, 5, 4, 4, 5, 5, 5, 6, 5, 4, 6, 4, 6, 4, 5, 6, 5, 4.
	W 8248	59	1	60	22, 8,	
	GH 8254	64	0	64	16, 17, 9, 21,	5, 5, 7, 13, 10.
	B 8321	43	0	43	9, 7,	
24% MILKFAT AND .4 GRAM YEAST DAILY <i>Second Generation</i> daughter of W 8187 (See text table XXVII)	W 8913	50	0	50	10, 5, 5, 5,	5, 5, 5, 6, 6, 4, 5, 4.
24% MILKFAT AND 1 GRAM YEAST DAILY (See text table XXVI)	B 8162	47	0	47	8, 7, 8, 7,	13, 14, 5, 12, 9, 6, 4, 6, 4, 6, 4, 4, 4, 5, 4, 4, 4, 4.
	G 8173	42	0	42	7, 6, 5, 6,	4, 5, 5, 5, 4, 4, 4, 4, 4.
	W 8218	48	0	48	9, 8, 7, 6,	4, 5, 5, 4, 4, 8, 4, 4, 4, 6, 5, 5, 4, 4, 4, 4, 6, 5, 5.
	W 8220	66	8	74	6, 8, 10, 8,	7, 5, 5, 5, 5, 4, 4, 4, 4, 4, 4, 4, 4.
	W 8231	69	15	84	22, 5, 11, 6,	5, 6, 5, 4, 6, 4, 4
	W 8241	45	0	45	10, 7, 15, 29,	5, 6, 5, 5, 6, 5, 5, 4, 4, 4, 4.
	W 8244	51	3	54	13, 7, 6, 5,	11, 10, 7, 6, 5, 4, 4, 4, 4, 4, 4, 4, 4, 4.
	W 8249	54	0	54	4, 7, 4.	
	B 8255	48	0	48	5, 5, 6, 5,	4, 4, 4, 4, 5, 6, 4, 6, 4, 4, 6, 5, 4, 4, 4, 4, 4, 3, 7, 4.
	B 8322	46	0	46	10, 7.	
	BH 8775	45	0	45	13, 8, 9, 11,	8, 5, 6, 4.
	B 8776	42	0	42	11, 5, 4, 6,	4, 4, 4, 6, 6, 5, 5, 5, 6.
24% MILKFAT AND 1 GRAM YEAST DAILY <i>Second Generation</i> daughters of B 8322 (See text table XXVII)	B 8777	44	0	44	13, 8, 7, 12,	9, 8, 7, 5.
	B 8778	45	0	45	12, 8, 6, 13,	6, 9, 8, 4.

APPENDIX TABLE VI—Continued

Single Added Foodstuff or Other Modification of the Basic Diet	Designa- tion of Rat	Age in Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina and First Oestrus	Age in Days at First Oestrus	Length in Days of First Four Cycles	Length in Days of Subsequent Cycles
9% CODLIVER OIL (See text table XXVIII)	W 8906	48	6	54	6, 5.	
	W 8912	63	16	79		
	GH 8934	55	2	57	5.	
25% YEAST AND 25% CASEIN (See text table XXX)	B 8930	46	1	47	7, 6, 5.	
	BH 8932	38	0	38	12, 5, 5.	
	W 8935	49	0	49	6, 6.	
18% LACTALBUMEN (See text table XXXI)	W 8908	48	0	48	11, 7.	
50% CASEIN (See text table XXXII)	W 8907	47	0	47	5, 5, 4, 4.	
	BH 8926	53	0	53	6, 6, 6.	
CYSTINE IN AMOUNT OF 0.5% OF PROTEIN (See text table XXXIII)	BH 9125	43	0	43	13, 4, 4, 4.	
	BH 9127	46	4	50	5, 4, 4, 4.	
CYSTINE IN AMOUNT OF 2% OF PROTEIN (See text table XXXIII)	W 9129	49	3	52	5, 5, 4.	
BASIC RATION WITH .4 GRAM YEAST DAILY (See text tables XXV and XXIX)	G 8170	65	0	65	7, 13, 5, 5, 6.	
	W 8186	59	3	62	15, 8, 8, 5.	
	W 8246	34	3	37	5, 4, 4, 4.	4, 4, 4, 4.
	GH 8252	36	0	36	5, 6, 5, 4.	6, 7, 4, 7, 9, 5, 4, 3, 6, 6, 4, 4, 4, 5, 5, 5, 5, 5, 5.
	W 8167	46	0	46	7, 10, 6, 11.	7, 10, 6.
	W 8172	58	1	59	41, 26, 8, 10.	16, 6, 16, 4.
	W 8233	59	0	59	14, (bred resorption)	4, 6, 4, 6, 5, 5, 4, 5, 5, 4, 4, 4, 4.
	B 8161	60	0	60	6, 5, 16, 6.	6, 4.
	W 8215	44	10	54	9, 13, 10, 8.	5, 10, 6, 5, 4, 5, 5, 4, 4, 5, 5, 6, 4, 4.
	W 8239	45	14	59	11, 11, 21, 6.	8, 9, 6, 5, 5, 5, 4, 5, 5, 5.
BASIC RATION WITH 1 GRAM YEAST DAILY (See text tables XXVI and XXIX)	W 8247	49	0	49	8, 7, 7.	
	GH 8253	36	0	36	7, 6, 6, 8.	5, 4, 6, 5, 5, 5, 5, 7, 4, 8, 5, 4, 5, 5, 5, 6, 5, 5, 5, 6.

APPENDIX TABLE VI—*Continued*

Single Added Foodstuff or Other Modification of the Basic Diet	Designa- tion of Rat	Age in Days at Rupture of Vaginal Closing Membrane	Number of Days Between Opening of Vagina and First Oestrus	Age in Days at First Oestrus	Length in Days of First Four Cycles	Length in Days of Subsequent Cycles
BASIC RATION WITH 1 GRAM YEAST DAILY	W 8165	56	7	63	9, 8, 10, 8,	7.
	W 8168	50	0	50	5, 6, 5, 5,	5, 5, 4, 6, 5, 5.
	W 8174	48	0	48	12, 10, 14, 6,	5, 12, 13, 5, 5,
						3, 6, 12, 8, 4, 4,
	W 8188	45	1	46	7, 9, 5, 5,	8, 5, 5, 4, 6.
	B 8195	37	0	37	6, 7, 8, 4,	6, 6, 5, 5, 5.
	W 8224	42	0	42	5, 5, 13, 9,	5, 5, 6, 4, 6, 5, 3.
						8, 7, 6, 5, 5, 5, 5.
						4, 5, 6, 5, 5, 5, 5.
	W 8234	43	0	43	8, 14, 12, 4,	5, 5, 5.
						12, 11, 24, 6, 5,
						5, 7, 6, 4.

APPENDIX TABLE VII

Showing the reproductive performance of rats reared on the basic ration to which had been added in each instance a single natural food-stuff or in which the proportions of the constituents had been altered. (Text tables XVIII to XXXIII give summaries of this table. The occurrence of first oestrus and ovulation performance of these rats is given in Appendix Table VI.)

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Litter	NUMBER OF YOUNG		WEIGHT OF YOUNG (in Grams)		WEIGHT OF MOTHER (in Grams)		Notes
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	
LETTUCE—10 gms. Fresh leaves daily (See text table XVIII)	days											
	60	B 8859	*	*	*	10	6	4.9	32.5	209	196	
	60	B 8862	*	*	*	9	6	5.4	28.0	185	192	
	60	B 8863	*	*	*	6	0	5.3		224		
						(4 dead)						
	60	BH 8864	*	*	*	11	6	5.5	28.7	200	200	
RAW BEEF MUSCLE 5 grams daily (See text table XIX)	60	W 9059	*	*	*	10	6	5.2	24.0	174	174	
	60	W 9062	*	*	*	9	6	5.5	25.0	201	200	
	60	W 9063	*	*	*	9	4	5.3	32.1	217	213	
	60	W 9061	*	*	?					222		No young found; lost 48 gms. in 1 day.
	60	GH 8905	*	*	*	9	5	4.8	35.6	235	246	
50% WHOLE WHEAT (See text table XX)	60	W 8909	*	*	*	9	4	4.9	29.5	216	256	
	60	W 8925	*	*	*	(1 dead)						
	60					10	6	5.6	37.7	230	228	
	60	BH 8996	*	*	*	6	0	4.7		183		Young lived 8-10 days only.
	60	BH 8997	*	*	*	8	6	6.2	26.7	211	192	
FRESH WHOLE MILK 5 cc. daily (See text table XXI)	60	B 8890	*	*	0							
	60	547	*	*	0							
	60	407	*	*	0							
	60	466	*	*	0							
		W 7256	*	*	0							
	60	B 8902	*	0	0							
		B 8667	*	0	0							



APPENDIX TABLE VII.—Continued

Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.) Found	Litter	NUMBER OF YOUNG		WEIGHT OF YOUNG (in Grams)		WEIGHT OF MOTHER (in Grams)		NOTES
						At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	
FRESH WHOLE MILK 10 cc. daily (See text table XXII)	60	G 8892	W 6636	0	0							
			554	*								
			462	0								
		G 8893	403	0								
			545	*								
1/3 SKIM MILK POWDER (See text table XXIII)	60	GH 8904	545	0	0							
			W 7810	*								
			465	*								
		W 9074	W 8762	*								
		W 9094	W 7965	*	?							No litter found; 24 gm. drop in weight in 1 day.
ORANGE JUICE 8 cc. daily (See text table XXIV)	60	W 9095	139	*	0							
		W 9070	B 8667	*	0							
		B 8894	W 7669	*	0							
		W 8898	W 8840	*	0							
		B 8901	B 819	*	*			4.0		180		
9% CODLIVER OIL (See text table XXVIII)	60	W 8906	W 8793	*	0							
			W 7526	*	0							
		W 8912	BH 81	*	*	1	0	4.0		184		Young dead second day.
		W 8912	472	*	0							
		G 8934	W 7669	*	0							
25% YEAST AND 25% CASEIN (See text table XXX)	60	B 8930	W 09	*	*	4	0	4.9		190		Young gone third day.
		BH 8932	453	*	0							
		W 8935	BH 6575	*	0							
			W 6905	*	0							
			G 7818	*	0							
50% CASEIN (See text table XXXII)	60	W 8907	468	*	0							
			GH 7835	*	0							
		BH 8926	471	*	0							
			547	*	0							
		BH 9125	W 7862	*	0							
CYSTINE IN AMOUNT OF 0.5% OF PROTEIN (See text table XXXIII)	60	BH 9127	W 8793	*	0							
				*	0							
CYSTINE IN AMOUNT (See text table XXXIII)	60	W 9126	W 8733	*	0							
				*	0							

	Designation of Female	Age at Breeding	Designation of Male	Specimen or Copulatory Plug Found	Placental Sign (r. h. c.) Found	Litter	Number of Young		Average Weight of Young (in Grams)		Weight of Mother (in Grams)		Autopsy Notes				Notes
							At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora	Foetuses	Resorption	Average wt. of Foetuses (in gms.)	
18% Lactation MPS (See text table XXXI)	W 8908	60 days	W 8707 B 8795 B 8667	• • •	• • •	0											
	W 8161	3 mos.	BH 56 W 55 BH 46 W 35 W 39	• • • • •	• • • • •	•	1 (dead)				217		1	6	0	1	2
			BH 7175 BH 7795	• • •	• • •	•	3	3	6.0	17.6	270	266					
	G 8171	3 mos.	W 82 W 35 W 8706	• • •	• • •	•	4	1	3.7	18.0	176	210					Killed 19th day of gestation 15 gram drop in weight found half eaten.
	W 8187	3 mos.	B 72	•	•	•	1	2	5.0	37.0	170	176					
	W 8214	5.5 mos.	W 91	•	•	•											
	W 8217	5.5 mos.	B 68	•	•	•	2	0	1.0		188						Young lived 3 days only
	W 8230	5 mos.	W 55	•	•	•	2 (dead)		6.0		186						
	W 8240	5 mos.	B 00 B 7460 540	• • •	• • •	•	6	0	4.8		228						Young lived 10 days
				• • •	• • •	•	4 (1 dead)	3	5.3	21.0	312	309					
	W 8248	3 mos.	B 90 B 68	• •	• •	•	2	0	4.5		192						Young lived 4 days. Killed 19th day of gestation
	GII 8254	5.5 mos.	W 09 W 6636 W 7256	• • •	• • •	•	2 (1 dead)	0	5.0		236						Young lived 1 day only

	Designation of Female	Age at Breeding	Designation of Male	Sperm or Copulatory Plug Found	Placental Sign (r. b. c.)	Litter	NUMBER OF YOUNG		AVERAGE WEIGHT OF YOUNG (in Grams)		WEIGHT OF MOTHER (in Grams)	AUTOPSY NOTES				NOTES
							At Birth	At Weaning	At Birth	At Weaning	At Littering	At Weaning	Corpora	Foetuses	Resorption	Average wt. of Foetuses (in gms)
9% MILKFAT AND 4 GRAM YEAST (See text tables XXV and XXIX)	B 8321	2 mos.	W 7307 W 00 W 6500 W 39 W 7473	* * * * *	0 0 0 0 0	* * * * *	3 0 3 4 5	0 5.0 6.0 4 5.6	18.3 240 252 16.8	296 240 252		R L R L R L R L				Young lived 19 days.
	G 8170	3.5 mos.	GH 35 B 66 W 8706 W 08	* * * *	0 0 0 0	0 0 0 0						5 5 0 0 0 3				Killed 19th day of gestation.
	W 8186	4 mos.	B 90 G 10 G 10	* * *	0 0 0	0 0 0						4 5 0 0 2 0				Killed 19th day of gestation.
	W 8246	2.5 mos.	W 94 B 8704	* *	0 0	0 0										
	GH 8252	5 mos.	W 5400 W 5400	* *	0 0	0 0										
	W 8167	3.5 mos.	W 93 W 04 GH 35	* * *	0 0 0	0 0 0						5 5 0 0 1 3				Killed 19th day of gestation.
	W 8172	6 mos.	B 00 W 09	* *	0 0	0 0										
	W 8233	2.5 mos.	B 68 W 66 W 45	* * *	0 0 0	0 0 0										
	B 8162	5 mos.	W 07 B 560	* *	0 0	0 0	6	5.7	26.1	276	272					35 gram drop in weight. No young found.
	G 8173	2.5 mos.	W 00	* *	0 0	0 0	0	3.4	202	248	236					Young lived 13 days
24% MILKFAT AND 1 GRAM YEAST (See text table XXVI)	W 8218	5.5 mos.	W 93	* *	0 0	0 0	7	6.0	27.3	248	236					

[illegible]

[illegible]

## OBSERVATIONS ON FASTING AND DIETS IN THE TREATMENT OF EPILEPSY

DAVID F. WEEKS, M.D., AND DAN S. RENNER, M.D.

*from the State Village for Epileptics, Skillman, New Jersey*  
and

FREDERICK M. ALLEN, M.D., AND MARY B. WISHART

*from the Physiiatric Institute, Morristown, New Jersey*

At the present time, when most subjects in medicine are being illuminated with rapidly increasing knowledge, it must be confessed that no ray of light has yet been shed upon the cause or treatment of various nervous disorders, of which epilepsy is a notable example. This disease constitutes an important public health problem. The three thousand registered epileptics in New Jersey are doubtless only a fraction of the real number in this state, and conservative authorities agree that fully 0.4 per cent. of the total population are thus afflicted in some degree. On this reckoning there must be nearly half a million epileptics in the United States. These alarming figures are not as widely known as they should be; and they, together with the hopeless and repulsive character of the affliction, should rouse interest in attempts at prevention, especially by eugenic measures, and in investigations to discover the origin and any possible improvements in the treatment of epilepsy.

The chief viewpoints up to the present have been, first, that epilepsy is purely a degenerative disorder of the nervous system, second, that it is either caused or excited by toxins, particularly from focal infections, and third, that it is either caused or influenced by internal secretory disturbances. To some extent the three hypotheses are mingled, but it may be noted that, except for attempts at palliation by drugs, the entire recent trend of therapy for epilepsy has been along the lines which are customary for metabolic disorders, namely, elimination of infectious foci, operations upon endocrine glands or administration of endocrine products, and dietary control. The surgical resections of organs such as the thyroid and adrenal, and the dosage with glandular products, particularly in the form of advertised proprietary preparations, have been so unfounded and likewise so fruitless that a review of this phase of the topic is unnecessary. Various



crude dietary regulations have been tried since early periods, but the recent interest on this point has been centered upon the influence of fasting.

With respect to published communications, priority must be given to Guelpa<sup>1</sup> for the advocacy of fasting for the routine treatment of epilepsy. The procedure was the same as employed by Guelpa for diabetes and the majority of known human ailments, namely, fasting of three days or slightly longer duration, with free saline purgation each day for eliminating supposed toxins, followed by a vaguely defined lacto-vegetarian regime. Guelpa's claims of benefits in epilepsy are even more indefinite than those in connection with diabetes. Details concerning methods of diagnosis, types of cases, and immediate and subsequent clinical results are lacking to a degree which prevents critical judgment of the work. It may be noticed that the treatment was not adopted in epileptic institutions or by physicians at large, but the same is true of Guelpa's diabetic treatment, which nevertheless contained a kernel of truth.

For some similar period of time H. W. Conklin, an osteopathic practitioner of Battle Creek, Michigan, has been using longer fasts for the treatment of epilepsy. There is no recorded proof whether this method was strictly original or was borrowed to any extent from Guelpa or others. A recent publication<sup>2</sup> outlines briefly the theory and application. Conklin here observes that 85 to 87 per cent. of the epileptics in public institutions have a history of either direct inheritance or great mental degeneration, or positive Wassermann tests, or a combination of several of these contributory causes, which are considered to be inimical to either the cure or the amelioration of such cases. These institutions, however, are said to hold only about 15 to 19 per cent. of the known epileptic population of their respective states, leaving more than 80 per cent. to be cared for at home. Conklin's patients are said to be drawn from this 80 per cent., and though the gross physical symptoms may seem identical, it is suggested that the causes and nature may be different. For the more curable type of the disease, without extreme nervous instability, great mental degeneration, or syphilis, Conklin proposes the term "intestinal epilepsy." He states, without details, that reasoning by elimination placed the seat of trouble in Peyer's glands, from which "we have been able in autopsies to extract a substance which injected into animals produces the same symptoms we find in epileptics." The large number of epileptic ex-soldiers applying to him for treatment is explained hypothetically as due to systemic poisoning by the large quantities of various serums used in the army. The storage of poison in the lymphatic systems of epileptics is said to be proved by observations that the removal of several ounces of blood, by producing a flow of lymph into the circulation, gives rise within 24 to 36 hours to exceptionally severe attacks. Without details of diagnoses or follow-ups, Conklin claims the following percentages of cures: Children of 10 years and under, 90 per cent.; 10 to 15 years, 80 per cent.; patients of 15 to 25 years, about 65 per cent.; from 25 to 40 years, 50 per cent., "and after

40 the percentage is very low." Failures are attributed, first, to the inability of nature always to restore normal functioning, and second, to secondary brain lesions that have been produced by the toxin in some cases. The treatment is based upon rest of Peyer's glands, relieving them of irritation, and stimulating their circulation by osteopathic treatment. All food is withdrawn and nothing but water given for as long a time as the patient can endure, ordinarily 18 to 25 days. Some crude tests for acidosis are mentioned, the most significant being a sudden fall of weight. It is indefinitely stated that the intestinal flora is changed by the fast. The long familiar increase of hemoglobin and red cell counts during fasting is mentioned as if it were a new discovery. Indigestion and constipation are said almost invariably to precede epilepsy. The importance of the patient's frame of mind is emphasized among the conditions for success, also the desirability of removing him from his home environment. Moderate exercise and recreation are encouraged during the fast. Headaches and nausea are included among the occasional fasting symptoms. The fast is broken with orange juice and water the first day, followed by gradual additions of liquid and solid food. Subsequent over-eating is to be avoided. Osteopathic treatments are prescribed during the fast for stimulating abdominal circulation, correcting "spinal acidosis," etc.

Geyelin was led to take up this study by personal observation of two young children who had numerous convulsions which were apparently cured or greatly relieved by Conklin's treatment. The only preliminary communication<sup>3</sup> which has been published merely summarized the findings concerning acidosis in some fasted patients. A paper was presented before the American Medical Association Convention in 1921 on the therapeutic results of the method, but, in view of the chronicity and variability of epilepsy, these were interpreted conservatively as only incomplete and publication has been deferred pending fuller experience. All that is available, therefore, is the abstract of this paper published in the Convention program, as follows: "The reasons for fasting in epilepsy. The method of conducting the fast. Results obtained over a period of one and one-half years in twenty-eight cases. (a) Clinical and therapeutic, (b) Acid excretions. Summary of important therapeutic procedure and results obtained with the present method described." In general, the method has followed that of Conklin, but the fasts are prolonged to three weeks or more only when convulsions do not cease sooner. Numerous failures or incomplete successes are acknowledged, but it is known that the results, including examples of complete freedom from symptoms up to two years following the treatment, have been considered so encouraging that the treatment has been at least tentatively adopted in several important clinics and is under study with regard to both its therapeutic and its scientific aspects.

Goldbloom<sup>4</sup> described a 10 year old girl who became free from petit mal attacks as long as she was in hospital and in bed. Fasting was employed for ten days, during which only a little clear broth was given. The diet was then gradually increased, but relapse occurred as soon as the child resumed her usual activities at home. Goldbloom entirely discredits the

fasting treatment, though the temporary relief in this case is nothing different from what Geyelin and his co-workers have admitted to occur in a considerable proportion of patients. Goldbloom further emphasizes the diagnosis. "When dealing with children we must first eliminate every other possible cause of convulsions before branding the child as an epileptic, for it is the neuropathic child and the one whose convulsions are purely of intestinal origin who are so apt to mislead us into drawing erroneous conclusions with regard to the efficacy of one or other form of treatment of true epilepsy."

Burr<sup>5</sup> has recently called fresh attention to the so-called spasmophilic diathesis, described by John Lovett Morse as follows: "Another common cause of convulsions, which is far more common than is usually appreciated in infancy and sometimes in childhood, is spasmophilia, a condition in which the normal balance between calcium and magnesium on the one side, and sodium and potassium on the other is disturbed, so that there is a relative diminution in the proportion of calcium and magnesium and a consequent increase in the irritability of the nervous system." From the records of 1654 Philadelphia cases, Burr substantiates the prevailing view that infantile convulsions are not necessarily forerunners of epilepsy. Also, when epilepsy later develops, "in many cases the interval between the first and second fit is so long, several years, that there is probably no relation between them."

The only experience of any of us with salts in relation to epileptiform attacks has been the single instance already reported<sup>6</sup> of a boy whose case had been diagnosed and unsuccessfully treated as *petit mal* and who was completely relieved by means of salt-free diet. Some negative observations on sodium chloride in relation to various nervous disorders will be reported later. As "spasmophilia" is a meaningless term, it may be important to determine more accurately the possible relation of high or low salt rations upon some such cases in children.

Our interest in the dietetic factor in epilepsy antedated any of the above publications. On the one hand, an influence of alimentary factors, indigestion, constipation, etc., upon the occurrence of epileptic seizures seems to be empirically established. Free saline catharsis is a routine measure in the treatment of severe attacks, and most institutions, including this Village, have followed some rules of diet regulation which were supposedly beneficial. On the other hand, the treatment of diabetes by fasting has inevitably suggested speculations concerning the possible usefulness of this method for other disorders. As it has never been demonstrated whether or not a poorly understood group of nervous disturbances, including epilepsy, are wholly or partly metabolic in nature, the purpose was formed several years ago

to investigate some of them as soon as opportunity permitted.

Suggestive arguments can be made for regarding epilepsy as a metabolic or chemical disturbance. The known hereditary element and the possible infectious influence are not unlike what is known for some metabolic diseases. In addition, there are, first, the absence of any regular or demonstrable organic cause of epilepsy; second, the progressive course, like that of a typical metabolic disorder, with either mild or severe onset but with a familiar sequence of aggravation and deterioration; third, the characteristic gluttony of epileptics, particularly of the lower type; fourth, the complete or partial relief of symptoms by fasting, as described by the above authors; fifth, the metabolic causation of epileptiform attacks in animals, of which the best known are the convulsions produced by meat feeding in Eck-fistula dogs. The hypothesis already exists that various nervous diseases may represent merely the irritation of a primarily healthy nervous system by abnormal substances originating elsewhere in the body.

If it be true that a temporary measure, like fasting, clears up the prominent symptoms of epilepsy, it must be regarded as surprising good luck if the benefit then continues through months or years of ordinary living and eating. The most important need would seem to be to determine, as in other metabolic diseases, what foods are chiefly responsible for trouble, so that a diet treatment may be devised which may not only be less onerous than fasting but also may be permanently carried out and perhaps thus control successfully some of the cases which have relapsed promptly after termination of fasting. The general failure of proposals like those of Guelpa can be readily understood; fasting can accomplish little in diabetes, for example, without accurate subsequent limitation of carbohydrate and calories, or in nephritis without appropriate restriction of protein and salt. Protein will perhaps fall first under suspicion as a cause of epilepsy. If intestinal processes are at fault, a diet of almost pure carbohydrate may change the intestinal flora and correct putrefactive processes more powerfully than fasting. If the intermediary protein metabolism is responsible, some such diet will reduce this metabolism lower than simple fasting. If the acetone bodies play a part, as suspected by Geyelin, carbohydrate feeding will abolish them, or high fat feeding will produce them in larger quantities than fasting. It is noteworthy



that all past attempts at epileptic diets have embodied merely vague ideas of non-irritating foods, or milk diet, or exclusion of meat, etc. Accurate trials of the possible specific influence of protein, carbohydrate and fat upon epilepsy are an entirely new undertaking.

This investigation was carried out in the autumn of 1921 as an earnest and hopeful endeavor to better the condition of epileptic patients. The methods were as follows:

*Selection of patients:* The purposes were explained to the families of a number of patients, and consent was thus obtained for trial of the dietary treatment in 73 individuals. The more intelligent patients also cooperated voluntarily, while the less intelligent ones were easily amenable. The list was made up so as to include a fair representation of the various types and grades of epilepsy represented in the Village, except the most extreme stages of severity and deterioration.

*Clinical care:* The greater part of the treatment was carried out in the hospital of the Village, which for a time was devoted exclusively to this work. Other groups of tests were conducted with the patients merely segregated in wards to which they were already accustomed. The entire clinical management was carried out by the regular staff of the Village, the most trustworthy and tactful nurses and attendants being chosen for this duty. Exercise was provided by allowing all the patients in the open air at fixed times daily, under the constant supervision of the staff. This supervision was continued day and night and all precautions were taken so that violations of diet were thoroughly excluded.

*Diets:* In addition to fasting, trial was made of diets representing respectively protein, carbohydrate, fat, high mixed rations, and non-nutritive bulk. These weighed diets were prepared by Miss Edith M. Shapcott of the Physiatrie Institute staff, who was transferred temporarily to the Epileptic Village for this purpose. As a rule, each patient went through two or three dietary regimes, so that comparisons were afforded between the different periods in the same individual, and also between the effects of different changes of regime. It may be stated here that the latter observations were negative, it being apparently immaterial whether a patient placed on a certain program had previously been on high protein, high carbohydrate, high fat, or on the regular mixed Village diet.

*Laboratory work:* The various analyses were performed by the staff of the Physiatrie Institute, partly in the Institute and partly in the Village. It was obviously impossible to carry on a wide range of analyses on such a large group of patients. Accordingly, it was planned to do a certain series of tests upon blood and urine samples taken at weekly intervals, and if possible continue such analyses daily throughout the entire closing week of each experimental period. This program was upset by unexpected recalcitrance of the patients regarding urine samples. Rubber urinals, which were provided as a precaution against losses during convulsions or at night, were persistently torn off by all lower grade patients. Daytime

collections from these patients were hopelessly unsatisfactory, and the higher grade patients, who were willing to cooperate, were nevertheless too careless for trustworthy results. After diligent trials of all the precautionary devices that could be thought of, it was concluded that accurate urinalyses on such a large group of patients were impossible. Quantitative work was therefore limited to the occasional blood analyses, which were always obtained without special difficulty or excitement. The correctness of the urine volumes as tabulated is questionable, and only qualitative tests were performed upon the urine.

It is somewhat remarkable that neither the routine tests nor the functional overload of the different diets revealed any of the ordinary metabolic diseases among these 73 epileptics selected at random. The experience in the Village indicates that diabetes, nephritis, hypertension, etc., are apparently uncommon among epileptics. The regular Village diet was found to be low in sodium chloride; the plasma chloride concentrations of the patients were low, and incidental variations of the chloride ration experimentally were without influence. A few determinations of blood urea and creatinin were also found normal.

The histories of the patients studied are given here in abbreviated summaries, sufficient to show their general character and type. Reference should be made to the large tables for the Wassermann reactions in blood and spinal fluid (negative except in 12 cases), and for the frequency of epileptic attacks before and after treatment. The entire group are classed as mental defectives in some manner or degree. It should be noticed nevertheless that some of the cases were of fairly mild epilepsy, and some of the patients might pass as mentally normal in a superficial or untrained examination. These psychiatric diagnoses, therefore, should not create confusion in comparison with any series of patients who have not been subjected to accurate psychiatric examination. Such records may serve an incidental purpose as an argument for effective legal measures to prevent the promiscuous breeding of such persons.

Case No. 1. Age 23. Italian. Occupation (never worked). High grade imbecile.

*Family history:* Maternal grandfather is said to have been insane. The patient's mother has had trifacial neuralgia, but otherwise the parents are in good health. The patient is ninth in line of birth of ten children, three of whom died before they were two years of age, one of them having had a convulsion before death.

*Personal history and examination:* Except for the ordinary childhood diseases, the patient has been healthy and rugged. He is a well developed male, 5 feet 2 inches tall, weighing 120 lb. Physical examination negative.

*Epileptic history:* The first convulsion is said to have occurred at the age of 11 months as the result of excessive and improper eating. The convulsions have since occurred at fairly regular intervals. He nevertheless made good progress in school and showed rather marked musical ability. Ordinarily he would classify as an Italian laborer.

*Treatment:* High fat diet for 48 days, followed by fasting for 3 weeks, then a return to regular Village diet.



Case No. 2. Age 29. American. Occupation (never worked). Medium grade imbecile.

*Family history:* The ancestry is supposedly healthy except for tuberculosis in a maternal grandmother. No cases of nervous or mental disease have been known, though the patient's mother is said to be of neurotic temperament. She has had four pregnancies, of which two resulted in stillbirths, one in a miscarriage, and the fourth in the birth of an apparently normal infant, the present patient.

*Personal history and examination:* He has been healthy except for the ordinary childhood diseases. He is a well developed male, 5 feet 9 inches tall, weighing 128 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 18 months without known cause. The attacks were at first some months apart, and development seemed to be fairly normal up to four years ago. Since then deterioration has been marked and the patient is now easily recognizable as a mental defective.

*Treatment:* High fat diet for 48 days, then fasting for 3 weeks, then return to usual Village diet.

Case No. 3. Age 35. American born. Occupation—laborer up to admission to Village. Medium grade imbecile.

*Family history:* Patient's parents are second cousins. The father is crippled and alcoholic. The mother is subject to nervous spells but has never lost consciousness during them. They had 11 children, of whom five are alcoholic. One sister had convulsions in childhood, and has five feeble-minded sons. Another sister also had convulsions in childhood. One brother was an epileptic and died at the age of 20 as the result of an accident. Another brother was insane and suffered with delirium tremens. One brother and one sister died during childhood. One brother and one sister appear normal. A maternal first cousin was an epileptic and had an epileptic son.

*Personal history and examination:* The patient has been healthy except for ordinary childhood diseases. He is a well developed male, 5 feet 8 inches tall, weighing 142½ lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 15 years from unknown cause. The patient was always of low mentality but has deteriorated very slowly. Ordinarily he might not attract notice among a number of laborers, but during his disturbed periods following convulsions he appears to have practically no intelligence.

*Treatment:* High carbohydrate diet for 24 days, then fasting for 3 weeks, then return to ordinary Village diet.

Case No. 4. Age 61. American. Occupation—laborer. Medium grade imbecile.

*Family history:* The father is said to have been alcoholic and extremely nervous. A paternal aunt was insane. There have been no known cases of epilepsy. The patient was fifth in line of birth of 11 children. The first five others died in infancy of unknown causes. The others have shown no symptoms of epilepsy.

*Personal history and examination:* The patient's general health has been excellent. He is a well developed male, 5 feet 4½ inches tall, weighing 143 lb. Physical examination shows mitral regurgitation.

*Epileptic history:* The first convulsion, at the age of 2½ years, was attributed to improper feeding. The attacks have been regular and frequent, commonly two or three in 24 hours. He has deteriorated greatly, has grandiose ideas, and at one time was committed to the Morris Plains State Hospital when in a state of mental depression with delusions of religious character. He is extremely violent following convulsions.

*Treatment:* High protein diet for 48 days, then returned to ordinary Village diet.

Case No. 5. Age 26. American born. Occupation—factory worker. High grade imbecile.

*Family history:* The parents are in good health. The patient's father, grandfather and grandmother were alcoholic. A paternal uncle and also a paternal aunt were epileptics. The patient represents the sixth of 14 pregnancies; there were 4 miscarriages, and 5 children died in infancy. One brother died of pleurisy at the age of 20; he is said to have had convulsions while teething. Another brother died at 14 of unknown cause. Two living sisters suffer from migraine.

*Personal history and examination:* Except for measles and eczema in childhood, the patient has always been healthy. He is a well developed male, 5 feet 1 inch tall, weighing 100 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 12 years from unknown cause. The attacks now come at fairly regular intervals, usually of the G. M. variety, seldom more than three in 24 hours. The patient would be taken for an ordinary laborer by the casual observer and is able to do considerable work, but he requires constant supervision.

*Treatment:* First a diet of non-nutritive bulky materials for 23 days, then high calory diet for 25 days, then return to ordinary Village diet.

Case No. 6. Age 24. American born. Occupation (never worked). Moron.

*Family history:* The patient's father, apparently normal, was killed in a railroad accident. A paternal grandfather was alcoholic. A maternal grandfather was insane. The patient's mother is migrainous. She had one epileptic sister. Six brothers of the patient are alcoholic.

*Personal history and examination:* The patient was delivered with instruments and was a blue baby. His childhood was uneventful except for accidents due to falls. He was quiet and worried a great deal. He is a well developed male, 5 feet 10 inches tall, weighing 137 lb. Physical examination negative.

*Epileptic history:* As a baby, the patient had "bad spells," in which he would become white around the mouth, and which his mother thought were due to heart trouble. He had no convulsions until after a fall from a baby carriage at 3 years of age. He then had five or six, and no more until he was 15 years old. Since then, the attacks have been rather frequent, of both G. M. and P. M. variety, with G. M. predominating and occasional status epilepticus. In disposition he is very sullen, disagree-

able, stubborn and contrary, but he otherwise shows little effects, and in the intervals between seizures he would not be picked out by a casual observer as a defective. His age in mental tests was placed at 11 years.

*Treatment:* High carbohydrate diet for 48 days, then fasting for 3 weeks, then return to Village diet.

Case No. 7. Age 31. American. Occupation (never worked). High grade imbecile.

*Family history:* Nothing is known of the grandparents. The mother was neurotic and died of cardiac disease. The father was a Yale graduate and a lawyer of exceptional ability. The patient has one brother who is said to have had convulsions and a criminal record but at present holds a very responsible position.

*Personal history and examination:* Nothing is known of his early childhood, or of any important diseases.. He is a rather frail male, 5 feet 4¾ inches tall, weighing 125 lb. Physical examination negative.

*Epileptic history:* When 8 years old, he fell from a hay loft, and shortly afterward began to have dizzy spells without loss of consciousness. He had no real convulsions until the age of 12. Since then, the attacks have been rather frequent, usually of G. M. variety, irregular in intervals and usually nocturnal. He has been able to travel a great deal about the country without attracting attention except when having convulsions. For the past two years he has shown considerable mental disturbance following convulsions, but during the intervals he would not attract notice as a defective.

*Treatment:* High fat diet for 48 days, then fasting for 3 weeks, then return to Village diet.

Case No. 8. Age 27. American. Occupation (never worked). Medium grade imbecile.

*Family history:* Nothing is known of grandparents. The father is normal, the mother very nervous. A sister of the patient is very nervous and a brother is feeble minded.

*Personal history and examination:* The general health has been good. Except for traumatic blindness of one eye, the physical examination is negative. The patient is a well developed male, 5 feet 4 inches tall, weighing 124 lb.

*Epileptic history:* At 4 years of age, the patient broke his leg and began to have convulsions shortly thereafter. Since then, he has had numerous G. M. and P. M. seizures and often is exhausted after a series. Up to a few years ago he showed considerable mental ability, but during the past four years he has deteriorated rather rapidly.

*Treatment:* First a diet of non-nutrient bulk for 23 days, then high calory diet for 25 days, then a return to Village diet.

Case No. 9. Age 17. American. Occupation—schoolboy. Moron.

*Family history:* Negative as far as learned. Both parents are in good health though the mother is somewhat neurotic. There have been 9 children (of whom the patient is sixth), in addition to seven miscarriages. Two brothers and one sister died in infancy of incidental causes. The other children show no signs of epilepsy but are more or less neurotic.

*Personal history and examination:* The patient has always been healthy and strong. He is a fairly well developed male, 5 feet 2 inches tall, weighing 96 lb. Physical examination shows myocardial disease.

*Epileptic history:* The first convulsion occurred at the age of 13 years, supposedly from overeating. At that time, he was attending school and making good progress. The attacks were at first several months apart but gradually became more frequent. The convulsions are usually in series and often completely exhaust the patient. During the intervals his mentality was good and he was able to hold a position in the Village printshop. During the past year he has not been able to recuperate to the same mental level as formerly.

*Treatment:* There was first a high fat diet for 24 days. As one of the usual series of convulsions then began, the patient was changed to high carbohydrate diet for 23 days. The series of attacks passed off in the usual manner without evident influence of the diet, but owing to this special condition the case is omitted from Table 6. The patient then fasted for 3 weeks, and afterward returned to the ordinary Village diet.

Case No. 10. Age 17. American. Occupation (never worked). Low grade imbecile.

*Family history:* Father in good health, mother tuberculous. No known cases of epilepsy or nervous disease. The patient is the oldest of eight children; all the others are delicate, anemic and show tendencies to tuberculosis.

*Personal history and examination:* Except for one attack of measles, the patient has always been well. He is a fairly well developed male, 5 feet 4 inches tall, weighing 115 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred without known cause at the age of 23 months. The attacks have continued frequently, and now often appear in series, especially at night. The mentality is very low.

*Treatment:* First a diet of non-nutritive bulk for 23 days, then a high calory mixed diet for 13 days, then a return to Village diet.

Case No. 11. Age 48. American. Occupation—editor. High grade imbecile.

*Family history:* The father had chronic asthma, and died from an accident. The mother was insane, and died of tuberculous meningitis at the age of 71. No epilepsy has been known in the family. The patient is the fourth of 6 children. The other five died of various causes not related to epilepsy.

*Personal history and examination:* The patient has been strong and healthy. He is a well developed male, height 5 feet 5½ inches, weight 136 lb. Physical examination is negative except for an old fracture of the left shoulder.

*Epileptic history:* The first convulsion occurred at the age of 26 years and was attributed to indigestion. The patient has been fairly successful as an editor of country papers, and the only peculiarity noticed has been a rather stubborn and disagreeable disposition. Other attacks followed the first at irregular intervals, free periods as long as 20 days being often followed by a series, during which he would become con-



trary, irrational and violent. At the time of this study he had deteriorated considerably and had many delusions, generally of a persecutory or grandiose nature.

*Treatment:* A high protein diet was given for 46 days. A series of seizures with the usual disturbed mentality then prevented continuance of the diet. The patient next fasted for 3 weeks, and then returned to ordinary Village diet.

Case No. 12. Age 15. Irish. Occupation—none. Moron.

*Family history:* The father is alcoholic, the mother and also her mother epileptic. A brother is feeble minded.

*Personal history and examination:* The patient had all the ordinary childhood diseases but otherwise has been well. He is a well developed boy, 4 feet 11½ inches tall, weighing 97 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 4 years without known cause. The subsequent attacks have been very irregular, sometimes as many as 400 in close succession, at other times as few as 6 per month. The mentality seems to have been very little influenced. He passed the Binet-Simon test for 8.9 years, and would appear to the ordinary observer as a rather bright schoolboy.

*Treatment:* High carbohydrate diet for 48 days, then fasting for 3 weeks, then return to Village diet.

Case No. 13. Age 21. American. Occupation—none. High grade imbecile.

*Family history:* Contains several cases of insanity and alcoholism but no epilepsy.

*Personal history and examination:* Patient has always been healthy. He is a well developed male, 5 feet 5 inches tall, weighing 114½ lb. Physical examination negative, except for hemiplegia.

*Epileptic history:* The first convulsion occurred at the age of 4½ years from unknown cause. The attacks returned, generally at fairly regular intervals, and sometimes in the form of series or status epilepticus. The mental development continued gradually, but not on a fully normal level. The patient would ordinarily be taken for a much lower grade individual than he is found to be after examination.

*Treatment:* High fat diet for 48 days, then fasting for 3 weeks, then return to Village diet.

Case No. 14. Age 22. American born negro. Occupation—schoolboy. High grade imbecile.

*Family history:* Nothing noteworthy. There has been no epilepsy or any other mental or nervous disease in the family.

*Personal history and examination:* The patient has always been healthy. He is a well developed male, 5 feet 7½ inches tall, weighing 135½ lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 15 years from unknown cause. Since then, convulsions occurred very frequently, usually two or three during 24 hours. He attends school, making good progress. Because of his kleptomaniacal tendencies, he requires constant

supervision. In an ordinary conversation, this boy would pass as a colored laborer.

*Treatment:* High carbohydrate diet for 48 days, then return to Village diet.

Case No. 15. Age 21. American. Occupation—laborer. Low grade imbecile.

*Family history:* There is a marked strain of alcoholism, feeble-mindedness and epilepsy on the paternal side, while on the maternal side there is a strain of insanity.

*Personal history and examination:* He has always been healthy with the exception of his epilepsy. He is a well developed male, 5 feet 6½ inches tall, weighing 139 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 3 years and was attributed to overeating. Since then the convulsions, of the G. M. variety, have occurred at fairly regular intervals, usually about 3 days apart. The boy's mentality did not develop with his physical growth. He is a low grade imbecile.

*Treatment:* High protein diet for 48 days, then fasting for 3 weeks, then return to Village diet.

Case No. 16. Age 32. American. Occupation—carpenter. High grade imbecile.

*Family history:* Patient's father was insane; otherwise the history is negative on both maternal and paternal sides.

*Personal history and examination:* Patient has always been healthy with exception of his epilepsy. He is a well developed male, 5 feet 9¼ inches tall, weighing 158 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 3 years. He developed mentally, and attended school where he made some progress. According to some of the mental tests, he has made a good average development, slightly above the normal. He has convulsions at irregular intervals, which usually occur singly and skip a period of 3 or 4 days, sometimes going as many as 24 days without a seizure. They are of the G. M. variety, and occur most often at night.

*Treatment:* Fasting for 3 weeks, then high calory diet for 25 days, then a diet of non-nutritive bulk for 23 days, then return to Village diet.

Case No. 17. Age 15. American. Occupation—none. Low grade imbecile.

*Family history:* There is a marked neurotic and epileptic strain on the maternal side. Health of parents is good.

*Personal history and examination:* The patient has always been healthy. He is a well developed male, 5 feet 3 inches tall, weighing 97 lb. Physical examination negative.

*Epileptic history:* The first convulsion, at the age of 1 year, was attributed to fright. Since then the attacks have occurred at fairly regular intervals. The boy is a low grade imbecile, never having developed mentally above four years.

*Treatment:* Fasting for 3 weeks, then high calory diet for 25 days, then a diet of non-nutritive bulk for 23 days, then return to Village diet.



Case No. 18. Age 10. American born Italian. Occupation—none. Low grade imbecile.

*Family history:* There is insanity and alcoholism on the paternal side, and alcoholism and tuberculosis on the maternal side.

*Personal history and examination:* Except the ordinary childhood diseases, he has always been healthy, but exhibited a rather troublesome, stubborn and sullen disposition. He is a well developed young boy, 4 feet 4 inches tall, weighing 69 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 18 months without known cause. He became very cunning, but otherwise showed very little mentality.

*Treatment:* High carbohydrate diet for 48 days, then return to Village diet.

Case No. 19. Age 18. American. Occupation (never worked). High grade imbecile.

*Family history:* Negative. So far as known, there is no epilepsy or other nervous or mental disease in the family.

*Personal history and examination:* Nothing can be learned of his personal history. He is a well developed male, 5 feet 3 inches tall, weighing 122 lb. Physical examination negative.

*Epileptic history:* The first convulsion, at the age of 3 years, was attributed to indigestion. His mentality did not develop in proportion to his physical growth and he never reached a stage of mentality higher than a high grade imbecile. He has convulsions at fairly regular intervals, seldom going 3 days without a seizure and frequently having 2 or 3 seizures during the day.

*Treatment:* High protein diet for 48 days, then return to Village diet.

Case No. 20. Age 34. American. Occupation—printer. Medium grade imbecile.

*Family history:* There is insanity and alcoholism on the maternal side of the family.

*Personal history and examination:* The patient suffered the ordinary diseases of childhood, and an injury of the spine at the age of 1 year. He is a well developed male, 5 feet 3½ inches tall, weighing 143 lb. Physical examination negative.

*Epileptic history:* The first convulsion, at the age of 5 years, was attributed to an injury of the spine. Convulsions recurred very frequently. However, he developed mentally, and as a young man was of fair mentality. For 10 years the patient has been deteriorating slowly and has now reached the stage of a medium grade imbecile.

*Treatment:* High protein diet for 48 days, then fasting for 3 weeks, then return to usual Village diet.

Case No. 21. Age 20. American. Occupation (never worked). High grade imbecile.

*Family history:* There is epilepsy and alcoholism on the paternal side, but the maternal side is negative.

*Personal history and examination:* With the exception of epilepsy,

the patient has always been healthy. He is a well developed male, 5 feet 2¾ inches tall, weighing 111½ lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 3 years without known cause. The seizures usually occur in series. The patient frequently goes 3 to 5 days without a convulsion, and seldom has more than three in 24 hours. His mentality has never been high. He has developed but not as rapidly as a normal person.

*Treatment:* Fasting for 3 weeks, then high calory diet for 25 days, then a diet of non-nutritive bulk for 23 days, then return to Village diet.

Case No. 22. Age 18. American. Occupation—schoolboy. Low grade imbecile.

*Family history:* Negative as far as learned. Parents of patient are normal and healthy.

*Personal history and examination:* The patient has always been sickly since birth, and suffered the ordinary diseases of childhood. He is a fairly well developed male, 5 feet 2 inches tall, weighing 106 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 9 months, and was attributed to dentition. Attacks continued very frequently, and his mentality did not develop with his years. He never reached a higher mental stage than that of a low grade imbecile.

*Treatment:* High protein diet for 48 days, then fasting for 3 weeks, then return to ordinary Village diet.

Case No. 23. Age 35. American. Occupation—machinist. Low grade imbecile.

*Family history:* There is insanity on both sides of the family, with a taint of alcoholism.

*Personal history and examination:* Except the ordinary diseases of childhood, and an injury to his head at the age of 8 years, which left no after-effects, the patient has always been healthy. He is a well developed male, 5 feet 3 inches tall, weighing 121 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 20 years without known cause. At this time he was a machinist and getting along very well at his trade. He deteriorated very rapidly, however, and at the time of the experiment showed very low mentality.

*Treatment:* High fat diet for 48 days, then fasting for 3 weeks, then return to Village diet.

Case No. 24. Age 27. American. Occupation (never worked). Medium grade imbecile.

*Family history:* Shows epilepsy and alcoholism on the maternal side of the family, and alcoholism is predominant on the paternal side. There is no epilepsy in the patient's immediate family.

*Personal history and examination:* The patient has always been healthy. He is a well developed male, 5 feet 4 inches tall, weighing 114 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 4 years without known cause. Since then, his convulsions, of the G. M. variety,

have continued at fairly regular intervals. He attended school and made fair progress, learning to read and write. At present he is able to do work about the house, but requires constant supervision because of his inclination to run away.

*Treatment:* High carbohydrate diet for 48 days, then return to ordinary Village diet.

Case No. 25. Age 24. American. Occupation—housework. Low grade imbecile.

*Family history:* Is negative as far as can be learned. The patient is third in line of birth of 5 children; the others are said to be normal.

*Personal history and examination:* The patient has always been healthy. She attended school but did not get along very well. She is an undernourished female, 5 feet 2 inches tall, weighing 115 lb. Physical examination negative.

*Epileptic history:* The patient fell when 2 years of age, injuring her head. Two weeks later she had a G. M. convulsion. Since then she has had G. M. and P. M. seizures at irregular intervals both day and night. She attended school, learned to read and write, and progressed very well. She would be taken by the ordinary observer for an ordinary girl working in a factory or some of the trades. She works very efficiently in the Village laundry.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 26. Age 46. American. Occupation (never worked). Low grade imbecile.

*Family history:* Shows alcoholism, feeble-mindedness and insanity. The patient is third in line of 7 conceptions; one child was feeble-minded, two died of convulsions during infancy, and there were three miscarriages.

*Personal history and examination:* Nothing is known of patient's early infancy. She is 4 feet 7½ inches tall, weighing 104 lb. Physical examination—mitral murmur, and short right leg due to old fracture.

*Epileptic history:* The patient has had convulsions since 2 years of age, and never reached a high grade of mentality. At the age of 6 years, her head was injured by a fall. She was unconscious for several hours and paralyzed for 9 months. Since that time her convulsions, of both G. M. and P. M. variety, have continued both day and night.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 27. Age 19. American negress. Occupation—housework. High grade imbecile.

*Family history:* Shows both alcoholism and feeble-mindedness.

*Personal history and examination:* Patient has always been healthy. She is a well developed female, 5 feet 1½ inches tall, weighing 132 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 2 years without known cause. She has had further attacks at irregular intervals, goes frequently for a period of 30 days without a seizure, and then is prone to have a series or status epilepticus. She has developed mentally, but not as rapidly as a normal child, and did not get along well at school.

She is a very good rough worker about the house, and would ordinarily be taken for an average colored servant.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 28. Age 15. American. Occupation (never worked). Low grade imbecile.

*Family history:* Shows alcoholism, insanity and feeble-mindedness. Patient has one feeble-minded brother.

*Personal history and examination:* The patient was a blue baby. Her childhood was uneventful, except scarlet fever at the age of 2 years. She is a poorly developed female, 4 feet 10 inches tall, weighing 71 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 9 months during teething. She has had convulsions at irregular intervals since that time, frequently going a month without a seizure. She frequently brightens up between convulsions, and is able to attend school and make some progress, but after a series of convulsions she is very much depressed and for a short time is untidy, taking absolutely no interest in her surroundings.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 29. Age 16. American Negress. Occupation (never worked). Medium grade imbecile.

*Family history:* Unobtainable.

*Personal history and examination:* The patient did not walk until 6 years of age, and gives history of chorea. She is an under-developed female, 4 feet 6 inches tall, weighing 87 lb. Except for rachitic chest, physical examination is negative.

*Epileptic history:* The first convulsion occurred at the age of 6 years without known cause. Since then, G. M. and P. M. convulsions have occurred at irregular intervals, usually as long as 7 to 10 days. She has developed mentally, made fair progress in school and learned to read. She is a good houseworker and under ordinary circumstances will rank with the average colored servant.

*Treatment:* Fasting for 3 weeks, then return to Village diet.

Case No. 30. Age 24. American. Occupation (never worked). Medium grade imbecile.

*Family history:* Shows insanity, epilepsy, feeble-mindedness and alcoholism.

*Personal history and examination:* Patient was a premature baby—7 months—and had a rupture at birth. She had scarlet fever at the age of 3 years. She is a poorly developed female, 5 feet tall, weighing 88½ lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 3 years. Her convulsions, of both the G. M. and P. M. variety, occur at irregular intervals, sometimes a period of several months elapsing without a seizure. She developed rather slowly, but learned to read and write. She is capable of doing considerable work, and works in the laundry, but is slow and of a mentality that requires supervision.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.



Case No. 31. Age 17. American. Occupation (never worked). High grade imbecile.

*Family history:* Shows epileptic, feeble-minded and alcoholic strains.

*Personal history and examination:* History of early childhood not available. She is a well developed female, 4 feet 11 inches tall, weighing 101 lb. Physical examination negative.

*Epileptic history:* The first convulsion, at the age of 7 years, was P. M. in character. Since then, she has had convulsions of both the G. M. and P. M. variety at irregular intervals, usually running a series when the convulsions start, and then going several days without a seizure. She shows considerable intelligence, assists with the care of other patients, and acts as an assistant to one of the teachers in school.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 32. Age 28. American born (German father, American mother). Occupation (never worked). Medium grade imbecile.

*Family history:* Not obtainable.

*Personal history and examination:* The patient was always healthy. She is a well developed female, 5 feet 8 inches tall, weighing 141 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 6 years, and seizures since then have been of both the G. M. and P. M. variety. She is often sullen and disturbed following convulsions. She is very moody at times; she will usually go about any task assigned to her in a cheerful manner and take care of the work, while at other times she refuses to do anything and appears not to understand directions.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 33. Age 15. American. Occupation (never worked). Low grade imbecile.

*Family history:* Shows alcoholic and feeble-minded taints.

*Personal history and examination:* The patient has always been healthy. She is a poorly developed female, 4 feet 7 inches tall, weighing 69 lb. Except for abrasions on the wrist caused by falling, the physical examination was negative.

*Epileptic history:* The first convulsion occurred at the age of 7 years, and both G. M. and P. M. seizures have occurred since, G. M. predominating. She made no progress in school, and has never developed mentally above the age of 4 years.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 34. Age 15. American. Occupation (never worked). Low grade imbecile.

*Family history:* Shows insanity and epilepsy.

*Personal history and examination:* With the exception of pneumonia at 2 years, the patient has always been healthy. She is a fairly well developed female, 5 feet 1 inch tall, weighing 87 lb. She shows an old dislocation of the right shoulder, with the joint very much relaxed; otherwise the physical examination was negative.

*Epileptic history:* The first convulsion occurred during teething. She then had none until 2 years of age, following pneumonia. Both the G. M.

and P. M. variety have occurred at irregular intervals since that time. She did not make good progress at school, but learned to read and write. She attends the Village school at present, but is making very little progress.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 35. Age 43. American. Occupation (never worked). Low grade imbecile.

*Family history:* Hereditary history not available. One of two brothers is said to be alcoholic. One sister said to be feeble-minded.

*Personal history and examination:* With exception of scarlet fever at 4 years, the patient has always been healthy. She is a well developed female, 5 feet 3 inches tall, weighing 117 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred during attack of scarlet fever at the age of 4 years. The attacks are of both G. M. and P. M. variety, the G. M. predominating. She is often disturbed after convulsions. She made poor progress at school. She developed very slowly, and has learned to read and write, but has been deteriorating rather markedly during the past five years.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 36. Age 46. German. Occupation—housework. High grade imbecile.

*Family history:* Very meagre. The father is said to have been alcoholic.

*Personal history and examination:* The patient has always been healthy. She is a well developed female, 5 feet 1 inch tall, weighing 127 lb. Physical examination negative.

*Epileptic history:* She had no convulsions until birth of her first child at about the age of 20 years, but was known to have had many hysterical laughing spells before that time. Since then G. M. attacks have occurred at irregular intervals. Sometimes she is badly disturbed following convulsions, and also suffers disturbed periods without relation to her convulsions. She has shown some mental deterioration, but has retained sufficient mentality that she would be taken for an ordinary foreign-born person of the laboring class.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 37. Age 19. American (German mother). Occupation (never worked).

*Family history:* Not available.

*Personal history and examination:* The patient had starts in her sleep during infancy. She is a well developed female, 5 feet 2 inches tall, weighing 77 lb. Physical examination negative.

*Epileptic history:* She has had convulsions, of the G. M. variety, since about 2 years of age. Following a seizure she is frequently disturbed and sometimes maniacal. She has frequent disturbed spells, and after each of these her mentality is slightly lower than before. She has reached the stage of deterioration which marks her as a mental defective.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.



Case No. 38. Age 21. American. Occupation (never worked). Low grade imbecile.

*Family history:* Shows epileptic strain.

*Personal history and examination:* The patient has always been healthy. She is a well developed female, 4 feet 9 inches tall, weighing 126 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 2 months. Since then the attacks have been rather frequent, being both G. M. and P. M. in character. The patient is very stubborn, especially after a series of convulsions, sometimes becoming violent. She has deteriorated so that she is now a low grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 39. Age 15. American. Occupation (never worked). Moron.

*Family history:* Shows alcoholic and neurotic strains.

*Personal history and examination:* The patient has always been healthy. She is a well developed, obese female, 5 feet 3 inches tall, weighing 145 lb. Physical examination negative.

*Epileptic history:* She had 7 G. M. convulsions at the age of 2 years, and then had no more until the age of 9, at which time they were severe and frequent. Shortly after her seizures began, she developed a very sullen, contrary disposition. She has convulsions at irregular intervals, sometimes daily, but frequently there is an interval of 7 to 10 days between seizures, followed by a series of 3 or 4. She attends school and is making very good progress. Her mental deterioration is very slight, and she would not likely attract attention if met casually.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 40. Age 16. American. Occupation (never worked). Moron.

*Family history:* Unobtainable.

*Personal history and examination:* The patient was always healthy. She is a well developed female, 4 feet 11 inches tall, weighing 104 lb. Her right eye is practically sightless; otherwise the physical examination was negative.

*Epileptic history:* The first convulsion occurred at teething, and there were no more until the age of 11 years. The seizures occur at irregular intervals, frequently 7 to 10 days without a convulsion followed by a series. At other times, her attacks occur daily and are usually of the G. M. variety. She is an exceptionally good looking girl, and her epilepsy would not attract attention among other girls of her own age.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 41. Age 29. American negress. Occupation (never worked). Low grade imbecile.

*Family history:* Shows insane and alcoholic strains.

*Personal history and examination:* No history of infancy is available. She is a dwarf female, 4 feet 4 inches tall, weighing 69 lb. Physical examination negative, except that she is rachitic, of the cretin type.

*Epileptic history:* It is not known when convulsions began. They occur at rather long intervals, usually of the G. M. type. She is a low grade imbecile dwarf.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 42. Age 21. American. Occupation (never worked). High grade imbecile.

*Family history:* Is not known.

*Personal history and examination:* Patient was healthy until 17 years of age. She is a well developed female, 5 feet 5 inches tall, weighing 101 lb. Physical examination negative.

*Epileptic history:* Shock of father's removal to the hospital is said to be responsible for the patient's first attack, at the age of 17 years. Her convulsions are always of G. M. variety, and she frequently has hysterical attacks in which she throws herself about the floor. She often becomes very maniacal. Her mentality is very good. She attended school, and made good progress. There has been some mental deterioration, and her conduct is now that of a mild mental defective.

*Treatment:* Fasting for 3 weeks, then return to regular Village diet.

Case No. 43. Age 23. Italian. Occupation—factory worker. Moron.

*Family history:* Shows alcoholic and epileptic strains.

*Personal history and examination:* Delivery of patient was very difficult. She is said to have led an immoral life. She is a well developed female, 4 feet 10½ inches tall, weighing 119 lb. Physical examination negative.

*Epileptic history:* The patient had convulsions during early infancy and childhood. They then ceased until the age of 12 years. The attacks are rather infrequent, often 10 days to 2 weeks apart, and are generally G. M. in character. Her appearance is good, and she shows little mental deterioration. She does good work in the laundry and sewing room, and would not be recognized as an epileptic unless seen during or immediately following a convulsion.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 44. Age 20. American. Occupation (never worked). Moron.

*Family history:* Shows alcoholic strain.

*Personal history and examination:* The patient has always been healthy. She is a well developed female, 4 feet 11 inches tall, weighing 118 lb. Physical examination negative.

*Epileptic history:* The convulsions began shortly after birth, and are of the G. M. variety. She frequently goes for a period of several months without convulsions, and often complains of headache after seizures. She attended school, making fair progress, and now acts as an assistant to the teacher in the sense-training class. Her mentality developed approximately in keeping with her physical development, and unless seen in a convulsion or shortly thereafter, she would not attract attention as being a mental defective.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 45. Age 32. Italian. Occupation—housework. Medium grade imbecile.

*Family history:* Shows alcoholic and feeble-minded strains. One sister was epileptic.

*Personal history:* The patient was always healthy. She is a well de-

veloped female, 5 feet  $\frac{1}{2}$  inch tall, weighing 104 $\frac{3}{4}$  lb. Physical examination negative.

*Epileptic history:* The first convulsions occurred at the age of 17, and were G. M. in character. She now has convulsions of both G. M. and P. M. types, which occur about every third or fourth day, and is sometimes disturbed after seizures. She has shown considerable deterioration and requires some supervision. She is easily recognizable as a mental defective by the casual observer.

*Treatment:* Fasting for 3 weeks, then return to regular Village diet.

Case No. 46. Age 22. Austrian. Occupation (never worked). High grade imbecile.

*Family history:* Is not known.

*Personal history and examination:* Patient was always healthy. She is a well developed female, 5 feet tall, weighing 97 $\frac{1}{2}$  lb. Physical examination negative, except laparotomy scar in the median line and an old dislocation of the right shoulder.

*Epileptic history:* The first convulsion, G. M. in type, occurred at the age of 3 years. The convulsions are now both G. M. and P. M. in character, and she is frequently depressed following seizures. She shows slight mental deterioration. She did housework, and unless seen in a convulsion or directly after, would not attract attention as being defective.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 47. Age 18. American. Occupation (never worked). Low grade imbecile.

*Family history:* Shows insane strain.

*Personal history and examination:* Patient had meningitis at the age of 6 weeks. She is an underdeveloped female, 4 feet 6 inches tall, weighing 62 lb. Physical examination negative.

*Epileptic history:* Convulsions began with attack of meningitis at the age of 6 weeks. The seizures have occurred at frequent intervals and are both G. M. and P. M. in character. She never developed higher than a low grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 48. Age 45. American. Occupation (never worked). Moron.

*Family history:* Heredity shows insanity.

*Personal history and examination:* The patient was always healthy. She is an underdeveloped, poorly nourished female, 5 feet 7 inches tall, weighing 87 lb. Physical examination—healed tuberculous lesions of the right lung; mitral murmur; endocarditis; old intracapsular fracture of the neck of the femur.

*Epileptic history:* The first convulsion occurred at the age of 35. Afterward her attacks occurred at increasingly frequent intervals, and were G. M. and P. M. in character. She was always quiet but rather stubborn. Her mental condition has deteriorated, but in ordinary conversation would not attract attention.

*Treatment:* Fasting for 3 weeks, then return to regular Village diet.

Case No. 49. Age 19. American. Occupation (never worked). Low grade imbecile.

*Family history:* Negative.

*Personal history and examination:* Patient was always healthy. She is underdeveloped and of anemic appearance, 5 feet 1 inch tall, weighing 105 lb. Physical examination negative.

*Epileptic history:* First convulsion occurred at the age of 5 years. The seizures have recurred at irregular intervals. She never developed above a low grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 50. Age 32. American. Occupation—none. High grade imbecile.

*Family history:* Shows alcoholic strain.

*Personal history and examination:* The patient was always healthy. She is a well developed female, 5 feet 2 inches tall, weighing 95 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 15 years. Attacks have recurred at rather frequent intervals until 5 years ago. Except that she is rather violent, irritable, and bad tempered, her mentality showed little effect. She has been slowly deteriorating and would now attract attention as a mental defective.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 51. Age 33. American. Occupation (never worked). Moron.

*Family history:* Negative.

*Personal history and examination:* The patient was always in good health. She is a well developed female, 5 feet  $\frac{3}{4}$  inch tall, weighing 113 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 8 years. Her mentality developed slowly and never reached a normal stage. She was capable of getting along at home and not attracting attention, except during or following a seizure. She has been deteriorating recently, and is recognizable as a defective.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 52. Age 37. English. Occupation—housework. High grade imbecile.

*Family history:* Shows epileptic strain.

*Personal history and examination:* The patient has always been healthy. She is a well developed female, 5 feet 1 inch tall, weighing 112½ lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 6 years. Since then she has had both G. M. and P. M. attacks at irregular intervals, frequently in series. During intervals her mentality is fair. She does considerable work about the house, but would attract attention as being mentally defective.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 53. Age 28. American. Occupation—factory worker. Moron.

*Family history:* Shows epileptic taint on both maternal and paternal sides. The patient has a sister who is neurotic.

*Personal history and examination:* Patient has always been healthy. She is a well developed female, 4 feet 11½ inches tall, weighing 118½ lb. Physical examination negative.



*Epileptic history:* The first convulsion occurred at the age of 14 years. Attacks are infrequent; she often goes 20 days without a seizure, and then has a series. Her mentality developed to a certain extent, so that until a few years ago she would not have attracted attention as mentally deficient among girls of her own age. Up to four years ago she was able to do fairly efficient work, was a member of the band, took leading parts in entertainments, etc., but since then she has deteriorated considerably.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 54. Age 20. American. Occupation (never worked). High grade imbecile.

*Family history:* All the patient's immediate family are epileptics.

*Personal history and examination:* The patient has always been healthy. She is a well developed female, 5 feet 3 inches tall, weighing 118½ lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 2½ years. Since then attacks have come at rather frequent intervals, usually not more than 1 attack in 24 hours, and she frequently goes 10 or 20 days without a seizure. There has been little mental deterioration, and she would not attract attention as a defective among girls of her age.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 55. Age 36. American. Occupation—none. Low grade imbecile.

*Family history:* One brother is feeble-minded; otherwise the history is negative.

*Personal history and examination:* The patient was always healthy. She is a well developed female, 4 feet 11½ inches tall, weighing 88 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 12 years. Attacks have recurred at irregular intervals. At first developed mentally, but has deteriorated to such an extent that she is now a low grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 56. Age 15. American. Occupation (never worked). Low grade imbecile.

*Family history:* Negative.

*Personal history and examination:* Patient has always been in good health. She is a well developed female, 5 feet 5½ inches tall, weighing 106 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 21 months, and attacks have returned at irregular intervals. Her mentality never developed above that of a low grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 57. Age 7. American. Occupation (never worked). Low grade imbecile.

*Family history:* There is a history of epilepsy on the paternal side; otherwise negative.

*Personal history and examination:* Patient has always been healthy. She is a well developed female child, 5 feet 4 inches tall, weighing 49 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 3½ years. Since then attacks have been very infrequent. Because of lack of training, she appeared to be of very low grade when admitted, but is slowly developing. She does not give promise of developing higher than a low grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 58. Age 31. American. Occupation (never worked). High grade imbecile.

*Family history:* There is a history of insanity on the maternal side, and alcoholism and insanity on the paternal side. There are no other epileptics in the family.

*Personal history and examination:* The patient suffered an injury to his head at the age of 15 years. Otherwise he has always been healthy. He is a well developed male, 5 feet 5½ inches tall, weighing 130 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 17 years, and was attributed to alcoholism. At that time his mentality was such that he would not attract attention, but he has deteriorated slightly during the past four years and would now be recognized as a mental defective.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 59. Age 42. American. Occupation (never worked). Medium grade imbecile.

*Family history:* Patient's mother died of tumor of the brain: otherwise the history is negative.

*Personal history and examination:* Patient had the ordinary diseases of childhood. He is a well developed male, 5 feet 1 inch tall, weighing 107½ lb. Physical examination reveals a hemiplegia of the left side with atrophy of left upper and lower extremities.

*Epileptic history:* The first convulsion occurred at the age of 2 years following sunstroke. The first attack lasted 2½ hours, following which hemiplegia of left side was noted. He has never developed mentally higher than a high grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 60. Age 28. Mixed negro and Indian. Occupation—farmer. Low grade imbecile.

*Family history:* Shows insanity, feeble-mindedness, epilepsy and alcoholism. All members of the family for generations are mixed negroes, Indians and whites.

*Personal history and examination:* He had the ordinary diseases of childhood. He is a well developed male, 5 feet 4 inches tall, weighing 120 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 14 years. His attacks are rather irregular. He has deteriorated very much and is now a low grade imbecile.



*Treatment:* Fasting for 3 weeks, then return to regular Village diet.

Case No. 61. Age 46. American. Occupation—silk weaver. Medium grade imbecile.

*Family history:* Negative except for slight strain of alcoholism in grandparents.

*Personal history and examination:* The patient had the ordinary diseases of childhood, including diphtheria and scarlet fever. He is a well nourished and developed male, 5 feet 3½ inches tall, weighing 111¼ lb. Physical examination negative.

*Epileptic history:* The first attack occurred at the age of 37 years while at work in a silk mill, without known cause. Since that time his convulsions have been very few, many months elapsing without a seizure. They are G. M. in type and depress the patient for several days. In the past two years he has been deteriorating rather rapidly, but during the interval of his convulsions he is capable of doing ordinary work, and would not attract attention as a mental defective among a gang of laborers.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 62. Age 16 years. American. Occupation (never worked). Moron.

*Family history:* Shows insanity and marked alcoholism on the paternal side. So far as known, there is no other epilepsy in the family.

*Personal history and examination:* The patient suffered the usual childhood diseases, and spinal meningitis at the age of 5 years. He is a well developed male, 5 feet 8 inches tall, weighing 159 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 5 years during an attack of spinal meningitis. The second seizure occurred 6 months later. The attacks are infrequent; there are intervals of 10 to 20 days without a seizure, generally followed by a series of two or three convulsions. Following an attack he is usually exhausted and depressed. His mentality has not developed fully with his age. He does splendid work in the print shop, and would not attract attention among other boys of his class.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 63. Age 26. American. Occupation (never worked). Medium grade imbecile.

*Family history:* Negative.

*Personal history and examination:* The patient was feeble-minded from birth. At the age of 16, he was committed to Randall's Island, where he remained for one year. He was then sent to the State Hospital for Insane at Morris Plains. He is a well developed male, 5 feet 6 inches tall, weighing 118½ lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at about the age of 17 years while in the State Hospital for Insane at Morris Plains. The attacks are very infrequent; he has been known to go three years without a seizure. He is slowly deteriorating, however, and is now a medium grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 64. Age 26. American. Occupation (never worked). High grade imbecile.

*Family history:* There is a slight strain of alcoholism on both sides of patient's family; otherwise the history is negative.

*Personal history and examination:* The patient suffered a fracture of the skull at the age of 11 years. He is a well developed and well nourished male, 5 feet 3 inches tall, weighing 134 lb. Physical examination negative, except an operative scar in the occipital region of the skull.

*Epileptic history:* His first convulsion occurred at the age of 18 months. The attacks have gradually increased in number and severity. His mentality developed slowly. He reached the mentality of a moron, but for the past few years has been slowly deteriorating, and would now be recognized as a mental defective by a casual observer.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 65. Age 20. American. Occupation (never worked). Moron.

*Family history:* Shows marked insanity and epilepsy.

*Personal history and examination:* The patient is a mute. He had the ordinary diseases of childhood, and, though puny at birth, developed into a healthy boy. He is a well developed male, 5 feet 1 inch tall, weighing 133 lb. Physical examination negative, except poor sight.

*Epileptic history:* The first convulsion, P. M. in character, occurred at the age of 12 years. Since then attacks have increased in frequency and severity. He attended the mute school and made good progress. He now works in the print shop and does good work, and takes considerable interest in the work and in his surroundings. Other than being a mute, he would not attract attention among people of his age.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 66. Age 44. American. Occupation—laborer. High grade imbecile.

*Family history:* There is a peculiar neurotic strain in the patient's family. His father suffered from migraine and his mother had peculiar mannerisms.

*Personal history:* Patient suffered the ordinary diseases of childhood. He is a well developed male, 5 feet 4 inches tall, weighing 125 lb. Physical examination reveals a partial ankylosis of the right elbow associated with an old fracture of the lower end of the humerus; otherwise the examination is negative.

*Epileptic history:* The first convulsion occurred at the age of 14, while swimming. A short time after his first seizure, he was struck on the head and was unconscious for a day, after which his convulsions increased in number and severity. He developed suicidal tendencies, but has shown none of these for several months. He works in the shoe shop, does very good work, but in an ordinary conversation would be recognized as a mental defective.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 67. Age 58. American. Occupation—longshoreman. Moron.

*Family history:* Not known.

*Personal history:* Patient was in State Hospital for Insane at Morris Plains for 1 year prior to admission here. He is a well developed male, 5 feet 9¾ inches tall, weighing 169 lb. Physical examination—cataract of the right eye, and deformity of left clavicle due to an old fracture; third finger of the right hand amputated at the second phalanx; depressed fracture scar, 1 inch by 1¾ inch in size, over right parietal bone.

*Epileptic history:* Patient states that he has had convulsions for many years, and that they followed a head injury. He has deteriorated slightly. He refuses to work, stating that he is an epileptic and the State must take care of him. He has some ideas of persecution, but otherwise would not attract attention in an ordinary conversation.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 68. Age 14. American. Occupation (never worked). Moron.

*Family history:* Not known, the patient having been admitted from an orphan asylum.

*Personal history and examination:* Apparently the patient has been in good health. He is a well developed male, 5 feet 4½ inches high, weighing 137 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 6 years. Since then attacks have occurred at infrequent intervals, usually in series. His mentality has developed sufficiently well that he would not attract attention among boys of his age.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 69. Age 40. American. Occupation (never worked). Moron.

*Family history:* Epilepsy on the paternal side.

*Personal history and examination:* The patient had paralysis of the right side, and the ordinary diseases of childhood. He is an over-developed male, 5 feet 7 inches tall, weighing 180 lb. Physical examination reveals marked paralysis of the right arm and forearm with contraction and atrophy; the right foot and leg show slight paralysis, probably an old hemiplegia.

*Epileptic history:* The first convulsion occurred at the age of 6 years, without known cause. He is hemiplegic and has never done any work. He is rather cunning and is one who utilizes his physical deformity for working on the sympathy of the public, and delights in telling stories of the many ways in which he has succeeded in obtaining money from strangers.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 70. Age 52. American. Occupation—farmer. High grade imbecile.

*Family history:* Shows alcoholism, insanity, epilepsy, feeble-mindedness and migraine.

*Personal history and examination:* Except for the usual diseases of childhood, the patient has always been healthy. He is a well developed male, 5 feet 6 inches tall, weighing 111 lb. Physical examination reveals an old healed tuberculous lesion in the right apex of the lung.

*Epileptic history:* The time of the first convulsion is not accurately known. He has fallen in the fire on several occasions, and carries many

scars and contractures as a result of falls. He has maintained his mentality, and except for a very contrary, sullen, egotistical disposition, would not attract attention in an ordinary conversation.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.

Case No. 71. Age 33. American. Occupation—printer. Low grade imbecile.

*Family history:* Not important.

*Personal history and examination:* The patient has always been healthy. He is a well developed male, 5 feet 4 inches tall, weighing 118 lb. Physical examination negative.

*Epileptic history:* The patient had "fainting spells" at the age of 14 years, but these were not regarded as convulsions. The first convulsion, G. M. in type, occurred at 19 years of age. Attacks have returned at rather infrequent intervals. His mentality developed so that he completed high school and was working as a printer before coming to the Village. After admission, he retained his mentality fairly well, until April 12, 1923, at which time he had status epilepticus, and since then he has been deteriorating rather rapidly.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 72. Age 29. Russian. Occupation—suitcase maker. Low grade imbecile.

*Family history:* As far as known, there is no epilepsy in the family.

*Personal history and examination:* Patient suffered the ordinary diseases of childhood including diphtheria; otherwise he has always been in good health. He is a well developed male, 5 feet 3 inches tall, weight 125 lb. Physical examination negative.

*Epileptic history:* The first convulsion occurred at the age of 1 year, and was G. M. in character. He has averaged 3 convulsions each month. He developed mentally and became a suitcase maker. He held the job for several years, but has deteriorated very markedly and is now a low grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to usual Village diet.

Case No. 73. Age 17. American. Occupation (never worked). Medium grade imbecile.

*Family history:* Reveals a marked strain of insanity, alcoholism and epilepsy on the paternal side, while alcoholism is predominant on the maternal side.

*Personal history and examination:* Patient had the ordinary diseases of childhood. He is a well developed male, 5 feet 3¾ inches tall, weighing 115 lb. Physical examination negative except for left lateral scoliosis of the spine.

*Epileptic history:* The first convulsion occurred at the age of 11 years, supposedly from a fall in which his skull was fractured. The fracture was operated on and several splinters of bone removed. Since that time he has had many convulsions and frequently becomes violent. During the intervals between seizures, he is peaceable and fairly quiet. He went to school, but his progress has always been poor. His mentality has never developed above a medium grade imbecile.

*Treatment:* Fasting for 3 weeks, then return to ordinary Village diet.





36	40	F	155	57	9	Neg.	8	6	2	3	0	0	0	2	1	1	0	50.9	1	1	8	7	450	Neg.	Neg.	Heavy	Neg.	70	Neg.	59.0	625	At beginning
																						425	Heavy	Heavy	Heavy	Neg.				End of 1st week		
37	19	F	153	35	3	Neg.	35	0	17	0	0	0	0	0	0	0	0	30	4	1	11	2	500	Mod.	Heavy	Neg.	115	Neg.	49.6	515	End of 2nd week	
																						250	Mod.	Heavy	Neg.					End of 3rd week		
38	21	F	145	57	2	4+	Neg.	16	5	2	3	2	0	0	0	0	1	50.2	8	2	26	3	350	Neg.	Neg.	Neg.	146	Faint	58.1		At beginning	
																						400	Neg.	Neg.	Neg.	146	Slight	57.2		End of 1st week		
39	15	F	160	65	9	Neg.	27	11	11	1	0	0	0	1	1	0	0	57.9	14	0	32	1	700	Mod.	Heavy	Neg.	70	Neg.	54.1	544	End of 2nd week	
																						200	Mod.	Heavy	Neg.	108	Neg.	47.5	544	End of 3rd week		
40	16	F	150	47	2	Neg.	39	17	5	2	2	1	2	0	4	2	33.1	3	4	25	8	1800	Slight	Heavy	Neg.	75	Neg.	35.3	570	At beginning		
																						400	Mod.	Heavy	Neg.	107	Neg.	38.2	573	End of 1st week		
41	29	F	132	31	3	Neg.	3	10	0	6	0	1	0	0	1	1	27.9	1	2	8	15	1150	Faint	Mod.	Heavy	Neg.	79	Neg.	46.2	533	End of 2nd week	
																						2000	Faint	Mod.	Heavy	Neg.	80	Neg.	58.6	589	End of 3rd week	
42	21	F	165	45	4	Neg.	30	11	12	6	1	0	0	1	0	0	40	11	0	42	8	450	Mod.	Heavy	Neg.	110	Neg.	60.1	518	At beginning		
																						600	Slight	Heavy	Neg.	107	Neg.	58.1	577	End of 1st week		
43	23	F	149	51	4+	Neg.	10	2	1	2	0	0	0	0	0	0	44.7	5	0	12	1	1200	Slight	Mod.	Neg.	93	Mod.	55.2	489	End of 2nd week		
																						250	Slight	Mod.	Neg.	112	Mod.	54.8	548	End of 3rd week		
44	20	F	150	53	6	4+	Neg.	1	0	1	0	2	0	0	0	0	45	0	3	3	3	550	Heavy	Heavy	Neg.	107	Neg.	57.7	573	At beginning		
																						1450	Slight	Mod.	Neg.	126	Mod.	54.6	501	End of 1st week		
45	32	F	153	47	6	Neg.	15	26	6	8	2	1	1	1	1	0	41.3	5	9	21	16	300	Neg.	Neg.	Neg.	86	Neg.	59.8	561	End of 2nd week		
																						300	Neg.	Faint—	Neg.	110	Neg.	58.6	538	End of 3rd week		
46	22	F	152	40	4	Neg.	15	10	1	2	2	0	1	3	3	0	30.5	18 days	0	0	0	0	600	Faint	Mod.	Neg.	82	Neg.	57.2	570	At beginning	
																						900	Mod.	Mod.	Neg.	117	Neg.	56.8	581	End of 1st week		
47	18	F	138	28	1	3+	Neg.	22	2	0	3	0	2	0	2	0	23.1	20	3	32	5	1300	Neg.	Faint—	Neg.	87	Neg.	57.2	489	End of 2nd week		
																						2000	Neg.	Neg.	Neg.	101	Neg.	57.8	550	End of 3rd week		
48	45	F	170	40	1	Neg.	8	6	0	2	10	1	0	0	0	1	25.1	1	2	.....	.....	1500	Faint	Mod.	Neg.	75	Faint	53.8	406	At beginning		
																						25	Faint	Mod.	Neg.	126	Faint	51.4	536	End of 1st week		
49	19	F	155	47	7	4+	.....	49	27	10	11	6	0	1	2	0	41.3	7	15	27	30	1000	Slight	Heavy	Neg.	75	Faint	40.8	519	At beginning		
																						250	Mod.	Heavy	Neg.	164	Faint	47.8	519	End of 2nd week		
																						450	Neg.	Neg.	Neg.	79	Neg.	59.1	556	End of 3rd week		
																						600	Mod.	Heavy	Neg.	99	Mod.	38.2	503	At beginning		
																						700	Mod.	Heavy	Neg.	104	Mod.	39.6	503	End of 1st week		
																						600	Neg.	Heavy	Neg.	104	Neg.	39.6	503	End of 2nd week		
																															End of 3rd week	





61	46	M	161	50.5	Neg.	Neg.	1	0	1	0	1	0	0	0	0	0	0	1100	Neg.	Neg.	Neg.	59.0	560	At beginning
																		300	Mod.	Neg.	Neg.	58.6	540	End of 1st week
																		325	Neg.	Neg.	Neg.	58.6	533	End of 2nd week
62	16	M	173	72.2	Neg.	Neg.	12	0	5	0	0	0	0	0	0	0	0	600	Faint	Neg.	Neg.	60.1	470	End of 3rd week
																		2900	Mod.	Faint	Neg.	60.1	568	At beginning
																		1870	Neg.	Neg.	Neg.	60.1	568	End of 1st week
																		3300	Neg.	Neg.	Neg.	52.3	520	End of 2nd week
63	26	M	168	54.2	Neg.	Neg.	0	0	0	0	0	0	0	0	0	0	0	250	Slight	Mod.	Mod.	54.2	520	End of 3rd week
																		1250	Neg.	Neg.	Neg.	58.5	552	At beginning
																		600	Neg.	Neg.	Neg.	58.5	552	End of 1st week
64	26	M	160	60.9	Neg.	Neg.	43	0	17	0	0	0	0	3	3	1	0	700	Neg.	Neg.	Neg.	56.4	570	End of 2nd week
																		900	Neg.	Neg.	Neg.	56.4	570	End of 3rd week
																		150	Neg.	Neg.	Neg.	56.7	557	At beginning
																		450	Neg.	Neg.	Neg.	56.7	557	End of 1st week
65	20	M	155	60	Neg.	Neg.	20	3	8	0	1	0	0	0	0	0	0	1090	Neg.	Neg.	Neg.	51.5	490	End of 2nd week
																		1650	Neg.	Neg.	Neg.	53.7	503	End of 3rd week
																		1450	Mod.	Mod.	Neg.	59.4	568	At beginning
																		2300	Neg.	Neg.	Neg.	49.8	500	End of 1st week
66	44	M	163	56.8	Neg.	Neg.	17	0	5	0	0	0	0	1	0	1	0	1300	Slight	Mod.	Neg.	48.0	552	End of 2nd week
																		750	Neg.	Neg.	Neg.	01.6	552	At beginning
																		1300	Faint	Faint	Neg.	58.3	590	End of 1st week
																		1200	Neg.	Neg.	Neg.	56.8	580	End of 2nd week
67	58	M	170.5	70.8	Neg.	Neg.	8	1	2	0	2	0	0	0	0	0	0	2075	Neg.	Neg.	Neg.	57.9	580	End of 3rd week
																		3000	Neg.	Neg.	Neg.	58.6	480	At beginning
																		3150	Neg.	Neg.	Faint	59.0	491	End of 1st week
68	11	M	161	62.2	Neg.	Neg.	0	0	4	0	4	0	0	0	0	0	0	275	Neg.	Neg.	Neg.	61.9	552	End of 2nd week
																		250	Faint	Mod.	Neg.	61.9	552	At beginning
																		1800	Neg.	Neg.	Neg.	56.3	553	End of 1st week
69	40	M	170	81.8	Neg.	Neg.	0	0	4	0	0	0	0	0	0	0	0	324	Neg.	Neg.	Neg.	54.8	510	End of 2nd week
																		800	Faint	Neg.	Neg.	58.2	500	At beginning
																		950	Neg.	Neg.	Neg.	58.2	500	End of 1st week
70	52	M	168	50.4	Neg.	Neg.	9	0	4	0	0	0	1	1	0	0	0	1400	Slight	Mod.	Neg.	50.7	619	End of 2nd week
																		1650	Neg.	Neg.	Neg.	50.7	619	At beginning
																		2100	Neg.	Neg.	Neg.	65.3	618	End of 3rd week
71	35	M	163	54.2	Neg.	Neg.	31	1	13	0	3	0	1	0	0	0	0	2200	Neg.	Neg.	Neg.	59.1	449	At beginning
																		1100	Neg.	Neg.	Neg.	56.9	540	End of 1st week
																		700	Faint	Faint	Neg.	51.5	486	End of 2nd week
																		225	Neg.	Neg.	Neg.	61.2	585	At beginning
72	20	M	160	56.8	4+	Neg.	10	0	10	0	4	2	0	0	4	1	0	900	Faint	Faint	Neg.	51.5	486	End of 3rd week
																		250	Neg.	Neg.	Neg.	61.2	585	At beginning
																		625	Neg.	Neg.	Neg.	56.1	510	End of 1st week
																		1200	Neg.	Neg.	Neg.	54.9	527	End of 2nd week
73	17	M	161.5	52.2	Neg.	2+	27	1	8	1	4	0	0	0	0	0	0	250	Slight	Mod.	Neg.	58.7	541	At beginning
																		220	Neg.	Neg.	Neg.	54.3	523	End of 1st week
																		100	Neg.	Neg.	Neg.	49.0	500	End of 2nd week
																		100	Neg.	Neg.	Neg.	49.0	500	End of 3rd week



11	48	M	166	52.2	Neg.	37	15	10	11	0	0	3	3	2	4	41.7	0	0	16	1	Neg.	Neg.	Slight	161	Neg.	515	At beginning
																				550	Neg.	Faint	130	Faint	507	End of 1st week	
																				670	Neg.	Faint	187	Faint	457	End of 2nd week	
12	15	M	181.5	43.1	Neg.	21	302	11	216	0	5	0	21	0	0	40.3	3	178	9	314	Neg.	Neg.	Slight	100	Faint	482	End of fast
																				1600	Neg.	Neg.	125	Neg.	556	At beginning	
																				1700	Neg.	Faint	106	Faint	511	End of 1st week	
																				1200	Neg.	Faint	80	Mod.	575	End of 2nd week	
13	21	M	165	50	Neg.	13	5	0	0	0	0	0	0	0	0	45.2	5	0	34	0	650	Mod.	Neg.	133	Faint	510	End of fast
																				.....	Heavy	Heavy	187	Faint	494	At beginning	
																				600	Mod.	Heavy	167	Neg.	511	End of 1st week	
																				2200	Heavy	Heavy	100	Faint	507	End of 2nd week	
15	21	M	169	55	Neg.	20	4	1	1	2	1	1	2	1	1	44.3	2	1	15	2	Neg.	Neg.	Neg.	131	Neg.	536	At beginning
																				.....	Neg.	Neg.	95	Neg.	536	End of 1st week	
																				200	Neg.	Neg.	93	Faint	474	End of 2nd week	
																				.....	Neg.	Slight	72	Faint	548	End of fast	
16	32	M	176	78.8	Neg.	11	5	3	0	2	0	2	1	2	0	69.7	0	0	12	7	Neg.	Neg.	Neg.	109	Neg.	474	At beginning
																				.....	Neg.	Neg.	111	Neg.	459	End of 1st week	
																				1700	Neg.	Faint	100	Faint	515	End of 2nd week	
20	34	M	161	55.9	Neg.	22	34	7	15	1	1	1	3	2	0	50.6	9	3	37	6	3250	Faint	Neg.	138	Neg.	491	At beginning
																				.....	Neg.	Neg.	118	Neg.	556	End of 1st week	
																				150	Faint	Mod.	125	Faint	478	End of 2nd week	
21	20	M	159.5	59.7	Neg.	33	32	8	8	7	1	0	0	6	0	51.2	8	12	24	32	Neg.	Neg.	Mod.	94	Faint	503	End of fast
																				.....	Neg.	Neg.	90	Neg.	581	At beginning	
																				1050	Neg.	Mod.	131	Neg.	491	End of 1st week	
																				450	Faint	Mod.	125	Faint	552	End of 2nd week	
																				1500	Slight	Mod.	76	Slight	552	End of fast	
22	18	M	158	45	Neg.	57	62	17	6	22	4	3	2	0	0	38.8	.....	Died	.....	850	Neg.	Neg.	130	Neg.	536	At beginning	
																				.....	Neg.	Neg.	91	Faint	578	End of 1st week	
																				.....	Faint	Slight	.....	Faint	520	End of 2nd week	
23	35	M	160	54.4	Neg.	20	3	3	1	1	1	2	1	3	1	47.2	2	0	13	0	1000	Slight	Mod.	102	Neg.	520	End of fast
																				510	Slight	Heavy	150	Faint	577	At beginning	
																				375	Faint	Heavy	155	Neg.	474	End of 1st week	
																				300	Neg.	Faint	113	Faint	571	End of 2nd week	
																				600	Neg.	V. Faint	.....	Neg.	571	End of fast	

TABLE III  
Observations With Non-Nutritive Bulk

Case No.	Age	Height cm.	Weight kg.	WASSER-MANN		CONVULSIONS								No. Days	Weight kg.	CONVULSIONS				Date, 1921	Diet	Urine Nitro-prusside	BLOOD PLASMA			
				Blood	Spinal Fl.	Preceding				Experimental Period						Following							Sugar mg. per 100 cc.	Nitro-prusside	CO <sub>2</sub> Vol. %	NaCl mg. per 100 cc.
						3 Months		1 Month		3 Months		1 Month				1 Month		3 Months								
						G.M.	P.M.	G.M.	P.M.	G.M.	P.M.	G.M.	P.M.			G.M.	P.M.	G.M.	P.M.							
5	26	155	45.4	Neg.	Neg.	58	16	7	16	12	4	23	41.8	6	11	37	12	Aug. 20	Village diet	Neg.	128	Neg.	56.8	471		
8	27	163	56.3	Neg.	Neg.	42	0	21	0	17	0	23	53.4	12	2	32	5	Sept. 8	Bulk	Faint	111	Neg.	48.5	527		
																		Sept. 23	Bulk	Neg.	121	Neg.	67.3	593		
																		Oct. 1	Bulk	Mod.	115	Neg.	58.2	491		
10	17	163	52.2	Neg.	Neg.	52	2	22	2	14	11	23	47.7	11	33	12		Aug. 25	Bulk	Heavy	106	Neg.	42.8	535		
																		Sept. 23	Bulk	Mod.	94	Neg.	74.9	533		
																		Oct. 1	Village diet	Neg.	136	Neg.	62.1	555		
16	32	176	71.8	Neg.	Neg.	15	5	4	5	3	0	23	69.2	1	0	10	1	Sept. 8	Bulk	Faint	97	Neg.	40.9	555		
																		Sept. 23	Bulk	Slight	117	Neg.	58.8	578		
																		Oct. 1	Bulk	Neg.	134	Neg.	56.0	535		
17	15	160	40.4	Neg.	Neg.	49	1	18	0	21	5	23	40.2	0	3	22	3	Aug. 25	Bulk	Mod.	127	Neg.	58.8	600		
																		Sept. 23	Bulk	Heavy	103	Neg.	47.5	556		
																		Oct. 1	Village diet	Neg.	91	Neg.	55.1	555		
21	20	159.5	50.4	Neg.	Neg.	48	21	15	12	9	12	23	46.8	8	7	35	25	Aug. 25	Bulk	Heavy	101	Neg.	65.1	535		
																		Sept. 23	Bulk	Neg.	130	Neg.	55.1	536		
																		Oct. 1	Bulk	Slight	182	Neg.	61.0	570		
				Neg.	Neg.							23	46.8	8	7	35	25	Aug. 26	Village diet	Neg.	125	Neg.	83.2	526		
																		Sept. 8	Bulk	Slight	.....	Neg.	48.5	536		
																		Sept. 23	Bulk	Mod.	123	Neg.	59.8	581		
																		Oct. 1	Bulk	Mod.	90	Neg.	.....	581		





TABLE V  
Observations With High Protein Diets

Case No.	Age	Height cm.	Weight kg.	CONVULSIONS				No. Days	CONVULSIONS				Date, 1921	DIET				BLOOD PLASMA									
				WASSER-MANN		Preceding			Experi- mental Period	Weight kg.		CONVULSIONS				Protein gm.	Fat gm.	C.H. gm.	Cal.	Urine Nitro- prusside	Sugar mg. per 100 cc.	Nitro- prusside	CO <sub>2</sub> Vol. %	NaCl mg. per 100 cc.			
												Following															
												3 Months		1 Month													
	Blood	Spinal Fl.	G.M.	P.M.	G.M.	P.M.	G.M.	P.M.	G.M.	P.M.	G.M.	P.M.															
4	61	164	65.0	Neg.	Neg.	26	60	7	28	7	6	48	59.1	4	27	26	32	Aug. 31	Village	11.0	0	562	Neg.	145	Neg.	58.0	538
11	48	166	61.3	Neg.	Neg.	38	1	11	1	14	8	46	58.6	4	6	12	7	Sept. 8	109.1	11.5	0	560	Neg.	130	Neg.	65.3	577
																		Sept. 23	111.0	11.4	0	560	Faint	136	Neg.	66.0	474
																		Sept. 30	132.8	11.4	0	562	Neg.	115	Neg.	61.5	515
																		Oct. 11	259.1	31.8	0	1323	Neg.	115	Neg.	61.5	515
15	21	169	63.1	Neg.	Neg.	30	2	10	1	6	2	48	57.9	5	6	12	7	Aug. 26	Village	14.0	0	562	Neg.	115	Neg.	61.5	515
																		Sept. 8	109.1	11.5	0	560	Neg.	124	Neg.	69.7	577
																		Sept. 23	114.0	11.4	0	560	Faint	161	Faint	48.1	515
																		Sept. 30	132.8	11.4	0	1323	Neg.	144	Neg.	56.9	474
19	18	160	55.9	Neg.	Neg.	60	8	18	7	27	6	48	51.3	13	0	43	0	Aug. 29	Village	14.0	0	562	Neg.	120	Neg.	59.5	527
																		Sept. 8	109.1	11.5	0	560	Neg.	111	Neg.	52.7	556
																		Sept. 23	114.0	11.4	0	560	Neg.	131	Neg.	48.1	538
																		Sept. 30	132.8	11.4	0	1323	Neg.	156	Neg.	60.8	538
20	34	161	65.0	Neg.	Neg.	41	23	7	16	12	12	48	60.2	6	12	28	16	Aug. 25	Village	14.0	0	562	Neg.	118	Neg.	58.3	654
																		Sept. 8	109.1	11.5	0	560	Neg.	122	Neg.	65.3	606
																		Sept. 23	114.0	11.4	0	560	Neg.	145	Neg.	62.4	536
																		Sept. 30	132.8	11.4	0	1323	Neg.	148	Neg.	62.4	536
22	18	158	48.1	Neg.	Neg.	48	60	24	35	36	29	48	51.3	28	0	Died	.....	Oct. 11	259.1	31.8	0	560	Neg.	147	Faint	67.1	646
																		Sept. 8	109.1	11.5	0	560	Neg.	138	Neg.	49.4	527
																		Sept. 23	114.0	11.4	0	560	Neg.	134	Neg.	57.5	527
																		Sept. 30	132.8	11.4	0	1323	Neg.	136	Neg.	64.6	554
																		Oct. 11	259.1	31.8	0	560	Neg.	96	Neg.	52.8	536

TABLE VI  
Observations With High Carbohydrate Diets

Case No.	Age	Height, cm.	WASSER-MANN				CONVULSIONS								Weight, kg.	CONVULSIONS				Date, 1921	DIET				BLOOD PLASMA						
			Blood		Spinal Fl.		Preceding				Experimental Period					No. Days	Weight, kg.	Following				Urine Nitro-prusside	Sugar mg. per 100 cc.	Nitro-prusside	CO <sub>2</sub> Vol. %	NaCl. mg. per 100 cc.					
							3 Months		1 Month		3 Months		1 Month					G.M. P.M.			G.M. P.M.						G.M. P.M.		G.M. P.M.		
																															G.M.
3	35	173	64.7	Neg.		30	4	3	4	4	2	24	62.7	0	0	14	10		Aug. 25	Village	diet	0	510	2116	Neg.	131	Neg.	62.5	552		
				Neg.		30	4	3	4	4	2	24	62.7	0	0	14	10		Sept. 8	19 0		0	440	1872	Neg.	103	Neg.		554		
6	21	178	62.2	Neg.		21	14	11	4	9	13	48	60.0	9	6	17	6		Sept. 30	28 0		0.5	263	1126	Neg.	125	Neg.	74.3	577		
				Neg.		21	14	11	4	9	13	48	60.0	9	6	17	6		Sept. 30	28 0		0.5	263	1126	Neg.	159	Neg.	58.9	494		
				Neg.		21	14	11	4	9	13	48	60.0	9	6	17	6		Aug. 8	19 0		0	510	2116	Neg.	101	Neg.		628		
				Neg.		21	14	11	4	9	13	48	60.0	9	6	17	6		Sept. 23	28 0		0	440	1772	Neg.	120	Neg.		597		
				Neg.		21	14	11	4	9	13	48	60.0	9	6	17	6		Sept. 30	11.4		0.5	269	1126	Neg.	113	Neg.	67.3	515		
12	15	151	40.4	Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Oct. 11	Village	diet	1.0	810	3398	Neg.	162	Neg.	58.2	318		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Aug. 31	Village	diet	0	510	2116	Neg.	158	Neg.		474		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 8	19 0		0	440	1872	Neg.	144	Neg.		587		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 23	28 0		0	440	1872	Neg.	125	Neg.	56.0	550		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 30	11.4	0.5	269	1126	3098	Neg.	126	Neg.	59.4	680		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Oct. 11	37.2		1.0	810	3098	Neg.	109	Neg.		433		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Aug. 26	Village	diet	0	510	2116	Neg.	115	Neg.		506		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 8	19 0		0	440	1872	Neg.	141	Neg.	57.0	515		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 23	28 0		0.5	269	1126	Neg.	132	Neg.	61.2	551		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Oct. 11	37.2		1.0	810	3398	Neg.	110	Neg.				
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Aug. 31	Village	diet	0	510	2116	Neg.	101	Neg.		581		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 23	28 0		0	440	1872	Neg.	101	Neg.	66.6	597		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 30	11.4	0.5	269	1126	Neg.	101	Neg.	57.8	577			
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Oct. 11	37.2		1.0	810	3398	Neg.	115	Neg.		554		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Aug. 31	Village	diet	0	510	2116	Neg.	122	Neg.				
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 8	19 0		0	440	1872	Neg.	109	Neg.		583		
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Sept. 23	28 0		0.5	269	1126	Neg.	109	Neg.				
				Neg.		30	32.7	7	58	7	108	48	44.7	1	140	8	381		Oct. 11	37.2		1.0	810	3398	Neg.	193	Neg.	63.6	412		

TABLE VII  
Observations With High Fat Diets

Case No.	Age	Height, cm.	Weight, kg.	CONVULSIONS				No. Days	Wgt. kg.	CONVULSIONS				Date, 1921	DIET			URINE		BLOOD PLASMA					
				WASSER-MANN	Preceding					Experimental Period	Following				Protein gm.	Fat gm.	C.H. gm.	Cal.	FeCl <sub>3</sub>	Nitro-prusside	Sugar Qual.	Sugar mg. per 100 cc.	Nitro-prusside	CO <sub>2</sub> Vol. %	NaCl. mg. per 100 cc.
					Blood	Spinal FL.	3 Months				1 Month	1 Month	3 Months												
1	23	138	51.3	Neg.	Neg.	36	1	8	1	21	11	48	51.8	Aug. 26	Village diet	1.0	1453	Neg.	Neg.	Neg.	Neg.	132	556		
														Sept. 8	16.1	152.1	2.5	2435	Neg.	Faint	Faint	125	527		
														Sept. 23	15.6	282.5	2.5	2435	Neg.	Mod.	Mod.	100	556		
														Oct. 1	21.0	245.3	2.5	2302	Neg.	Faint	Faint	120	593		
														Oct. 11	21.6	581.9	3.7	5365	Neg.	Faint	Faint	176	474		
														Oct. 25	22.4	439.8	3.5	4082	Neg.	Heavy	Heavy	166	597		
2	29	175	57.9	Neg.	Neg.	36	6	17	1	18	8	43	57.7	Aug. 24	Village diet	5.0	1453	Neg.	Neg.	Neg.	Neg.	135	412		
														Sept. 8	16.1	152.1	2.5	2435	Neg.	Slight	Slight	135	515		
														Sept. 23	15.6	282.5	2.5	2435	Neg.	Mod.	Mod.	161	515		
														Oct. 1	21.0	245.3	2.5	2302	Neg.	Heavy	Heavy	215	586		
														Oct. 11	21.6	581.9	3.7	5365	Neg.	Heavy	Heavy	250	556		
														Oct. 25	22.4	439.8	3.5	4082	Neg.	Heavy	Heavy	337	577		
7	31	165	59.7	Neg.	Neg.	35	4	12	4	8	3	43	59.7	Aug. 28	Village diet	5.0	1453	Neg.	Neg.	Neg.	Neg.	134	536		
														Sept. 8	16.1	152.1	2.5	2435	Neg.	Slight	Mod.	120	556		
														Sept. 23	15.4	282.5	2.5	2435	Neg.	Heavy	Heavy	198	597		
														Oct. 1	21.0	245.3	2.5	2302	Neg.	Faint	Faint	158	412		
														Oct. 11	21.6	581.9	3.7	5365	Neg.	Faint	Faint	156	474		
														Oct. 25	22.4	439.9	3.5	4082	Neg.	Mod.	Mod.	166	577		
9	17	158	43.1	Neg.	Neg.	137	156	62	51	20	89	24	42.7	Aug. 31	Village diet	5.0	1453	Neg.	Neg.	Neg.	Neg.	117	494		
														Sept. 8	16.1	152.1	2.5	2435	Neg.	Slight	Slight	150	597		
														Sept. 23	15.6	282.5	2.5	2435	Neg.	Mod.	Mod.	138	577		
														Oct. 1	21.0	245.3	2.5	2302	Neg.	Slight	Neg.	121	577		
														Oct. 31	Village diet	5.0	1453	Neg.	Neg.	Neg.	Neg.	113	578		
														Sept. 8	16.1	152.1	2.5	2435	Neg.	Slight	Slight	153	578		
														Sept. 23	15.2	282.5	2.5	2435	Neg.	Mod.	Mod.	182	579		
														Oct. 1	21.0	245.3	2.5	2302	Neg.	Neg.	Neg.	182	513		
														Oct. 11	21.6	581.9	3.7	5365	Neg.	Slight	Slight	160	536		
														Oct. 25	22.4	439.8	3.5	4082	Neg.	Mod.	Mod.	187	43.8		
														Aug. 25	Village diet	6.0	1453	Neg.	Neg.	Neg.	Neg.	152	61.8		
														Sept. 8	16.1	152.1	2.5	2435	Neg.	Slight	Mod.	121	515		
														Sept. 23	15.2	282.5	2.5	2435	Neg.	Heavy	Heavy	608	598		
														Oct. 1	21.0	245.3	2.5	2302	Neg.	Mod.	Mod.	125	568		
														Oct. 11	21.6	581.9	3.7	5365	Neg.	Mod.	Mod.	109	568		
														Oct. 25	22.4	439.8	3.5	4082	Neg.	Slight	Slight	171	577		
														Oct. 25	22.4	439.8	3.5	4082	Neg.	Slight	Slight	190	47.5		

*Remarks on Fasting (Tables 1 and 2)*

At the beginning of the investigation, 49 patients were changed from the regular mixed diet of the Village to complete fasting for three weeks, during which time nothing but water was given. (Table 1.) Meanwhile other patients were started on special weighed diets, as described below. At the close of these observations, 15 of the special diet group were started on fasting. (Table 2.) In this way, a total of 64 fasting experiments were carried out, all of three weeks' duration.

*Weight and strength:* The fall of body weight was moderate, partly perhaps because of the indolent habits of the patients. It ranged mostly between 5 and 10 kg. in different individuals for the three weeks. The rule which is well known for normal persons held good also in these patients; namely, hunger or other disagreeable feelings were trivial, and after the second day requests for food practically ceased and they behaved in their usual manner. In the majority of patients the strength was perceptibly but not seriously reduced. It returned promptly within a few days after eating was resumed, and no harm resulted from the fasting in any case.

*Laboratory findings:* The plasma sugar was maintained at an approximately even level, as usual in fasting. Acidosis was generally present but never severe. This statement is based upon the comparison of the nitroprusside and ferric chloride reactions in the urine, of which the former was generally moderate or heavy and the latter negative or slight. Furthermore, the nitroprusside reaction of the blood plasma was generally negative or faint, thus excluding any serious retention of acetone bodies. In the few instances in which moderate nitroprusside reactions appeared in the plasma, the  $\text{CO}_2$  capacity was usually not seriously reduced. In the 2 cases (Nos. 39 and 49) in which reductions of the  $\text{CO}_2$  capacity occurred below 40 volumes per cent., no clinical symptoms were noticeable, and neither interruption of the fast nor any special treatment was required. This course of events may be explainable by the fact that the patients drank water liberally in order to fill their stomachs, even when the urine volumes shown are small because of loss of part of the samples. In general, therefore, the patients reacted to fasting as might be expected of a similar group of normal persons, and without modification attributable to the epilepsy.

*Therapeutic results:* In 30 of the 64 instances, epileptic attacks remained absent during the final week or two of fasting. Continuance of fasting did not always improve the results; for example, in cases No. 21, 36, 37, 52, 72 and 73, attacks which had been absent reappeared during the closing days of the fast. In 16 instances (cases No. 7, 15, 16, 23, 25, 27, 28, 32, 47, 50, 51, 54, 58, 60, 70 and 73) fasting produced no change in the attacks, which were as frequent and violent as before the treatment. On the whole, however, glancing through Table 1 will show a perceptible reduction in the attacks during the fasting period. In general, after the termination of the fast the attacks returned the same as before. Any apparent exceptions to this rule in the tables can be easily matched by the spontaneous variations which have occurred at different times in the same or other cases in this Village. The clinical judgment was furthermore that the patients received no real benefit even during the fast. The partial subsidence of attacks appeared to be a merely incidental phenomenon, no different from the results of any indifferent procedure which produces as strong a psychic effect upon the patients as did the fasting period. The mental state was unimproved, and in a few cases of rapidly deteriorating type this deterioration seemed to continue unaltered by the fast. An opinion based merely upon our general experience must be that the patients were epileptic to just the same degree as before, even when their acute seizures were more or less suppressed under the temporary influence of the fast.

#### *Remarks on Bulk Diets (Table 3)*

As a control upon the above observations and interpretations, 6 patients were changed from the regular Village diet to one of non-nutritive bulk. The materials used for filling the stomach were the same as have been familiar in diabetic treatment, namely, thin soup, agar jellies, thrice-cooked green vegetables, bran extracted with boiling water, celluloflour, gum arabic, mineral oil, petrolatum, etc., cooked in various ways and flavored with spices, vinegar, artificial extracts, etc. These patients were good subjects for such a diet, because they took it readily in large quantities without knowing the lack of food value. Some psychic effect was present, because of the special environment and guarding, the radical change from the former diet, and the less pleasing character of the new menus. This diet prevented intestinal rest



and provided a maximum of irritating material in the mechanical sense. These observations were continued for 23 days.

*Weight and strength:* The fall of body weight was surprisingly slight, approximately 5 kg. at the maximum, and only 0.2 kg. in case 17. The smallness of the changes is perhaps explained to a slight extent by digestion of the bran and vegetables, but chiefly by the weight of the material in the intestine and by salt and water retention, though edema was not perceptible. The strength was reduced to somewhat the same degree as by plain fasting.

*Laboratory findings:* The plasma sugar fell slightly, but never to subnormal levels. Acidosis was trivial in all cases, the differences from simple fasting being supposedly due to the small carbohydrate digestion, and the salts obtained from the vegetables and bran.

*Therapeutic results:* It may be said that changes were absent in cases 8 and 16 and doubtful in case 10. In the other three cases the attacks did not cease, but were reduced in somewhat the same fashion as by plain fasting. The slightly less striking effects are probably explained by the slighter psychic impression. The influence upon subsequent seizures was approximately the same as with fasting. The most noteworthy observation seems to be the complete absence of perceptible harm from the very bulky and mechanically irritating diet.

#### *Remarks on High Protein Diets (Table 4)*

Six patients were transferred from the mixed Village diet to the highest possible protein rations, which were continued for 48 days. Beginning with 110 gm. protein and 14 gm. fat, as a precaution against harm, the rations were rapidly increased to 260 gm. protein and 32 gm. fat, which was the largest quantity that the entire group of patients would regularly eat. This diet consisted of clear soup, the leanest kinds of meat and fish, egg white and casein, being thus low in fat and free from carbohydrate. With thorough cooking, such diets are smooth and relatively non-irritating mechanically, but they furnish a maximum of hypothetical nitrogenous toxins and a maximum influence in the direction of a putrefactive intestinal flora. The psychic effect was comparatively slight, because the patients were pleased with the large quantity and variety of meats, and with casein cookies did not particularly miss bread or other carbohydrates.



*Weight and strength:* Undernutrition was present, because the highest calories on this program amounted to no more than 1325 daily. In this respect the experiment was a partial fast, and all the patients lost weight except No. 22, who was the smallest in size. The loss of weight in some of these cases in 48 days of reduced diet was as great as in certain individuals in tables 1 and 2 during their three weeks of complete fasting. The bodily strength was preserved better than in the fasting group.

*Laboratory findings:* The plasma sugar showed no marked or consistent changes. Acidosis was absent or negligible.

*Therapeutic results:* In the first three cases tabulated, attacks occurred less frequently during the experimental period than in the succeeding period. In the last three cases, attacks were more frequent during the experimental period than before. The after results seemed to be entirely variable. All the effects seemed to be of the same incidental or accidental character as with the other diets. The most impressive result was the apparent absence of harm from the long period of high protein feeding.

#### *Remarks on High Calory Diets (Table 5)*

These observations are related to the above two topics, because the six patients at the end of their regime of non-nutritive bulk were changed quickly to the largest quantities they could eat, both of protein and of other food. This forced feeding continued for 25 days in five instances and for 13 days in one instance. Full sway was given to the characteristic epileptic gluttony, which was augmented by the preceding hunger. These diets averaged not less than 175 gm. protein, 530 gm. fat, 700 gm. carbohydrate, and over 8000 calories. Some individuals at times took over 200 gm. protein and over 9000 calories. Indigestion and constipation were absent or easily controlled, and the only psychic effect was one of great satisfaction and contentment.

*Weight and strength:* With their indolent lives, these patients gained weight at an astonishing rate, and their fatness at the end of the experiment was in striking contrast to their condition at the beginning. The bodily strength also increased greatly.

*Laboratory findings:* The plasma sugar rose, as would be expected in contrast to the former inanition, but the forced feeding with all classes of food did not drive it above approximately normal limits.

*Therapeutic results:* The frequency of the seizures varied entirely accidentally, being sometimes less and sometimes greater than in the preceding and following periods. The most important observation is the absence of any apparent aggravation of the epilepsy by the most excessive feeding.

*Remarks on High Carbohydrate Diets (Table 6)*

Six patients were transferred from the Village mixed diet to the highest possible carbohydrate, which was continued for 24 days in one instance and for 48 days in five instances. This diet consisted to the greatest possible extent of corn starch and cane sugar, with enough flour, cereals, and high carbohydrate vegetables and fruits to prevent distaste. These diets furnished from 500 to 800 gm. carbohydrate daily. The protein only exceptionally rose as high as 37 gm., and frequently was no more than 11 gm. per day. Fat was either absent or below 5 gm. daily. The willingness of the patients to eat anything set before them was helpful as usual.

*Weight and strength:* The changes in body weight were slight and variable. The reduction of strength was not very noticeable.

*Laboratory findings:* Acidosis was naturally absent. On the whole, the high and one-sided carbohydrate diet failed to raise the plasma sugar.

*Therapeutic results:* The frequency of seizures again seems to vary accidentally, in relation to the comparative absence of psychic effect. These high carbohydrate diets may be expected to change the intestinal flora from a putrefactive to a fermentative type as far as possible, and at the same time to reduce protein products, derived either from the bowel or from intermediary metabolism, to a decidedly greater degree than fasting. The absence of benefit from such a program is the chief lesson.

*Remarks on High Fat Diets (Table 7)*

Six patients were taken from the mixed Village diet and placed on a high fat regime for 48 days. As a precaution, this change was made gradually, as follows:

Date. 1921	Protein gm.	Fat gm.	C.H. gm.	Calories
Sept. 8 to 23	15	260	2.5	2410
Sept. 24 to Oct. 1	21	250	2.5	2344
Oct. 2 to 11	21	580	3.7	5318
Oct. 12 to 26	22	440	3.5	4062

These performances are without parallel in the literature of one-sided diets. One of the writers has published a preliminary communication<sup>6</sup> on attempts of this kind in dogs, and will give fuller details later. Irrespective of the presence or absence of ketosis, dogs, pigs and all other laboratory species, as well as human beings, have developed absolute disgust to such overbalanced fat diets, involving complete refusal of food, indigestion, and toxic symptoms if the diets were forced. It is hard to say which was the most remarkable, the eating, the digesting, or the metabolizing of such diets by the epileptics. No normal or diabetic person could continue to enjoy eating a piece of butter the size of a man's fist one or more times daily through such a period, and the same applies to the drinking of salad oil. Fecal analyses were not performed but the solid consistency and small amount of the stools gave proof of uniformly good digestion. Only toward the close the appetite began to flag slightly, and it will be noticed that the fat had to be reduced somewhat in the final period for this reason.

*Weight and strength:* The weight changed very little, though it is probable that fat was deposited and water driven out in a manner not shown by the scales. The strength and general comfort were only slightly reduced.

*Acidosis:* Acidosis was surprisingly slight. The urine always showed positive nitroprusside reactions, but the ferric chloride reaction remained negative in two cases. The nitroprusside reaction of the blood plasma indicated only a slight retention, and the plasma bicarbonate was surprisingly little changed. It is difficult to judge whether this behavior is peculiar to epileptics, as normal persons could scarcely go through such a program. It seems probable, however, that both normal and abnormal individuals may vary widely with respect to ketogenesis and are not necessarily governed by fixed rules. In addition, all these patients except No. 3 at the close of the high fat period were placed on absolute fasting, and showed no special tendency to acidosis because of the previous one-sided ration.

*Plasma sugar:* Every patient gradually developed marked hyperglycemia in consequence of the high fat diet. This was determined by the Benedict method with and without charcoal, and by the Folin-Wu method. The highest figure reached was 0.357 per cent. in case 2. Glycosuria was absent, the high renal threshold being perhaps comparable to that in diabetics when

the hyperglycemia is brought on by fat feeding.<sup>7</sup> Such hyperglycemia in these non-diabetic subjects was the more remarkable, since nothing of the sort had been produced by high carbohydrate, protein or high calorie mixed diets. The sugar fell to normal in the five patients who were transferred to absolute fasting, and also in patient No. 3 who was transferred directly to high carbohydrate. These observations were unique until a similar rise of sugar was noticed by Atkinson,<sup>8</sup> though the fat stuffing in his experiments was less extreme.

*Therapeutic results:* The frequency of seizures seemed to vary accidentally. The attacks did not cease in any instance, and there was no demonstration of either harm or benefit in the immediate or subsequent results.

### *Conclusions*

1. The therapeutic value of fasting was tried in epileptic patients for three weeks. Epileptic seizures ceased during the fast in only 30 out of 64 instances, but in general the observations confirm those of previous writers that the attacks are more or less diminished during this time. We have interpreted this result as an accidental phenomenon, such as can easily be produced in epileptics by a variety of psychic and other influences, and not as a genuine therapeutic benefit.

2. This interpretation based on clinical experience is strengthened by the unchanged progress of the epilepsy after termination of the fast, and also by the effects of special diets. If fasting really benefits epilepsy, it was assumed as a corollary that one or more forms of food must be harmful to epilepsy. Diets of non-nutritive bulk, high protein, high carbohydrate, high fat and high total calories were found to be neither harmful nor beneficial.

3. These diet tests have afforded no indication of a metabolic element in epilepsy. On the practical side, the most important conclusion is that any kind of healthful mixed diets may be used in the management of epilepsy.

4. The group used for this study has included various degrees and stages of epilepsy. Any criticism directed against the character of the cases must be based on some demonstration of a radical division in epileptic types. Our observations cannot disprove a speculation that certain cases are inherently milder, or that they are functional rather than organic, and therefore

respond differently to fasting. Such a cleavage in diagnosis and therapy has not yet been recognized by authorities on epilepsy or proved by existing evidence. We believe rather that a permanent cure by fasting casts doubt on the diagnosis of epilepsy.

5. The most important observations theoretically were the astonishing tolerance of these patients for one-sided fat diets, without serious acidosis or clinical symptoms, and still more the remarkable hyperglycemia which developed in every case on high fat diet and not with any of the other diets. It seems desirable that persons having the proper equipment should take advantage of the unusual opportunity offered by epileptic patients, by studying a small group of cases with accurate urinary and respiratory analyses.

#### REFERENCES

1. Guelpa, G., and Marie, A. *Bull. gén. thérap.*, 160, 1910, 616-624. La lutte contre l'épilepsie par la désintoxication et par la rééducation alimentaire. Cf. also Guelpa, *Autointoxication and Disintoxication*, translation by F. S. Arnold, New York, 1912; also earlier journal articles.
2. Conklin, W. H. *J. of the American Osteopathic Association*, Sept., 1922. Cause and treatment of epilepsy.
3. Geyelin, H. R. *Amer. Soc. for Clinical Investigation*, 1921.
4. Goldbloom, A. *Canadian Med. Assn. J.*, 12, 1922, 539-540. Some observations on the starvation treatment of epilepsy.
5. Burr, C. W. *Archives of Pediatrics*, 39, 1922, 301-307. The relation of infantile convulsions to epilepsy.
6. Allen, F. M. *Amer. J. Physiol.*, 42, 1916-1917, 583-584. Observations concerning fat feeding.
7. Allen, F. M., and Wishart, Mary B. *J. Biol. Chem.*, 43, 1920, 129-147. The renal threshold for sugar and some factors modifying it.
8. Atkinson, H. V. *J. Metabol. Research*, 1, 1922, 565-607. The transformation of protein into fat and fat into carbohydrate in the body.



# THE DISTRIBUTION OF SULFUR IN PROTEIN-FREE-MILK.

By BARNETT SURE AND R. E. O'KELLY.

*From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville.*

In connection with his studies on the nutritive value of lactalbumin the senior author stated that protein-free-milk contains the greater part of its sulfur in the organic form.<sup>1</sup> In this paper quantitative analytical data are presented to bear out that statement.

In this investigation three samples of protein-free-milk from three different breeds of cows were used. These samples were prepared according to the following technique: Two to three liters of skimmed milk were diluted four to five times with distilled water, and a 10 per cent. solution of acetic acid slowly added with vigorous stirring until the precipitation of casein was complete. The casein was then filtered through cheese cloth and the filtrate refiltered through filter papers. The casein whey was then heated to a temperature of 80 degrees C. and kept at that temperature for about 15 minutes until all the lactalbumin coagulated. A clear filtrate was thus obtained, which on further heating gave no more cloudiness. The lactalbumin whey was then allowed to evaporate to dryness at room temperature with the aid of an electric fan, and a white powder was obtained which was further dried in the electric oven at a temperature of 70 degrees C. The material was then placed in a desiccator and used when needed for analysis. The samples prepared gave the following nitrogen content:

<i>Breed of Cow</i>	<i>Holstein</i>	<i>Ayrshire</i>	<i>Jersey</i>
Per Cent. Nitrogen	0.52	0.47	0.52

Preliminary trials showed a total sulfur content of about .13 per cent. and an inorganic sulfate content of about .02 per cent.

## *Methods of Analysis.*

*Determination of Inorganic Sulfates:* One to two grams of protein-free milk was dissolved in about 400 c.c. distilled water, filtered and slightly acidified with HCl, and a solution of 5 per cent. barium chloride was added in the cold drop by drop until precipitation was complete. The barium sulfate precipitates were allowed to stand for 24 hours and then filtered, after thorough washing with distilled water, through tarred gooch crucibles.

*Determination of Total Sulfates:* One to two grams of protein-free milk was dissolved in 200 c.c. distilled water, filtered; then 4 c.c. concentrated HCl were added and the solution allowed to boil for 30 minutes.



The heating was carried on in Ehrlenmeyer flasks covered with crucible covers, so as to allow the reflux hydrolysis of ethereal sulfates to proceed without any loss of water. The solution was then diluted to 400 c.c. and a 10 per cent. solution of barium chloride added to the hot solution until precipitation was complete, and the barium sulfate was filtered the next day through tarred gooch crucibles. Varying the amount of HCl in the concentration of the solution employed did not alter the nature of the results in any significant degree. Such slight variations were obtained that they were considered negligible.

*Ethereal Sulfates:* The ethereal sulfates constitute the additional amount of sulfur obtained after hydrolysis, and are represented by the difference between the total sulfates and the inorganic sulfates.

The methods employed for the analysis of inorganic and total sulfates are essentially those devised by Folin<sup>2</sup> for urine analysis. The only modification introduced is the concentration which had to be worked out for the conditions of solution and precipitation in connection with the protein-free-milk material.

*Determination of Total Sulfur:* After a number of preliminary trials the following fusion method was found to yield optimum results in connection with the total sulfur determination in protein-free-milk. The sample was slightly moistened with water in a nickel crucible, 5 grams sodium carbonate added, and the mixture stirred to a stiff paste. Five grams of sodium peroxide were then slowly added, and the material was ignited until melted, and then further heated to a solid mass. After being allowed to cool a little it was then spread with 4 grams additional sodium peroxide. The sample was then ignited until melted, after which it was heated for about 15 to 20 minutes. It was then cooled, and distilled water added while still fairly warm until a volume of about 400 c.c. was obtained. Four cubic centimeters of concentrated HCl were then added and boiled until clear. To the clear acid solution 5 c.c. of dilute alcohol (1 part of ethyl alcohol to 4 parts of H<sub>2</sub>O) were then added to expel the last traces of chlorine, and the boiling then continued for 15 minutes. A 10 per cent. solution of barium chloride was then added to the hot solution until precipitation was complete, and the barium sulfate filtered the next day through tarred gooch crucibles.

TABLE I  
Showing Sulfur Content of Synthetic Milk Salt-Mixture.

Kind of Sulfur	Weight of Sample* (Grams)	Weight of BaSO <sub>4</sub> Ppt. (Grams)	Weight of Sulfur (Grams)	Per Cent Sulfur
Total Sulfates.....		None		
Total Sulfur.....	1.3137	.0034	.000465	.036
	2.0244	.0062	.000348	.042
			Average	.039

\*In the weight of sample are included the weights of K<sub>2</sub>SO<sub>4</sub> and cystine.

TABLE II

Showing recovery of different forms of sulfur from a mixture of  $K_2SO_4$  and cystine added to a sample of a synthetic milk salt mixture where the percentage of sulfur in the inorganic form was 0.026 and the percentage in the organic form was 0.104, making a total sulfur content of 0.130 per cent.

Form of Sulfur	Weight of Sample (Grams)	Weight of $BaSO_4$ Ppt. (Grams)	Weight of Sulfur (Grams)	Per Cent Sulfur
Inorganic.....	0.9710	.0018	.000241	.025
Sulfate.....	1.0929	.0019	.000259	.024
Total.....	1.0305	.00121	.000165	.161
Sulfur.....	1.5799	.00187	.000254	.161

In order to obtain the above distribution of the different forms of sulfur, it was calculated that it was necessary to add 0.00112 gm.  $K_2SO_4$  and 0.003896 gm. cystine per gram of the milk salt mixture.

The amounts of sodium peroxide and sodium carbonate indicated above are given for a one gram sample of protein-free-milk. Whenever the sample employed exceeded the one gram weight the amounts of the above reagents were increased proportionately.

The reagents were found to be very pure and absolutely sulfur free. The synthetic milk salt mixture, however, contained some organic sulfur, which is indicated in Table I.

*Organic Sulfur:* Organic sulfur represents the difference between the total sulfur and the total sulfates.

In order to prove for ourselves that the salts in protein-free-milk—particularly the phosphates—do not interfere with the direct precipitation of sulfates, several trials were made incorporating inorganic sulfate material in the form of  $K_2SO_4$  and organic sulfur material in the form of cystine in various proportions. Both the  $K_2SO_4$  and the cystine gave the theoretical yields of sulfur by the direct precipitation in the cold, and the fusion method and precipitation in the hot, which showed these substances to be chemically pure.

According to Söldner<sup>3</sup> the salts in milk are present in the following proportions:

Sodium Chloride .....	10.62	per cent.
Potassium Chloride .....	9.16	" "
Monopotassium phosphate .....	12.77	" "
Dipotassium phosphate .....	9.22	" "
Calcium citrate .....	5.47	" "
Dimagnesium phosphate .....	3.71	" "
Magnesium citrate .....	4.05	" "
Dicalcium phosphate .....	7.42	" "
Tricalcium phosphate .....	8.90	" "
Calcium citrate* .....	23.55	" "
Lime combined with casein.....	5.13	" "

100.00 per cent.

\* The authors examined Söldner's original article and found that calcium citrate appears twice in his table and his figures are, therefore, employed as given in his paper.

TABLE III

Showing Recovery of Different Forms of Sulfur from a Mixture of  $K_2SO_4$  and Cystine Added to a Sample of a Synthetic Milk Salt Mixture Where the Percentage of Sulfur In the Inorganic Form was 0.104 and the Percentage In the Organic Form was 0.026, Making a Total Sulfur Content of 0.130 per Cent.

Form of Sulfur	Weight of Sample* (Grams)	Weight of $BaSO_4$ Ppt. (Grams)	Weight of Sulfur (Grams)	Per Cent Sulfur
Inorganic.....	0.7999	.00735	.00101	.103
Sulfate.....	1.1231	.00970	.00132	.117
Total.....	1.2440	.0155	.00204	.162
Sulfur.....	1.0451	.0122	.00167	.165

In order to obtain the above distribution of the different forms of sulfur, it was calculated that it was necessary to add 0.00567 gm.  $K_2SO_4$  and 0.00097 gm. cystine per gram of the synthetic milk salt mixture.

TABLE IV

Showing Distribution of the Various Forms of Sulfur In Protein-Free-Milk Taken from Three Different Breeds of Cows.

Sample No.	Breed of Cow	Form of Sulfur	Weight of Sample (Grams)	Weight of $BaSO_4$ Ppt. (Grams)	Weight of Sulfur (Grams)	Per Cent Sulfur
I	Holstein	Inorganic Sulfate	1.4165	.0023	.000316	.022
			1.2395	.0017	.000233	.019
		Total Sulfate	1.6715	.0048	.000663	.040
			1.5040	.0046	.000635	.042
		Total Sulfur	1.8312	.0191	.00262	.143
			1.5182	.0155	.00213	.140
II	Ayrshire	Inorganic Sulfate	1.1692	.0015	.000206	.017
			1.9339	.0023	.000315	.016
		Total Sulfate	1.4587	.0028	.000381	.026
			1.2917	.0024	.000332	.026
		Total Sulfur	1.5308	.0115	.00158	.103
			1.5500	.0121	.00166	.106
III	Jersey	Inorganic Sulfate	2.1631	.0026	.000357	.016
			0.9043	.0015	.000206	.022
		Total Sulfate	1.2459	.0041	.000569	.046
			1.2560	.0042	.000572	.046
		Total Sulfur	1.9217	.0187	.00257	.134
			2.0111	.0202	.00277	.137

\*In the weight of sample are included the weights of  $K_2SO_4$  and cystine.

TABLE V

Showing the Large Percentage of Organic Sulfur In Protein-Free-Milk.

Sample No.	Breed of Cow	Total Sulfur	Total Sulfate	In-organic Sulfate	Ethe-real Sulfate	Or-ganic Sulfur	Per Cent of Organic Sulfur of Total Sulfur
I	Holstein	.141	.041	.021	.020	.100	71.5
II	Ayrshire	.110	.026	.017	.009	.084	76.4
III	Jersey	.135	.046	.019	.027	.089	65.9

The salt mixture used in this work was, therefore, made up according to Söldner's findings, omitting the last item "lime combined with casein," since that would introduce additional foreign organic sulfur from the casein. The omission of that item altered the percentage of the salts of milk, which was recalculated on the basis of 100 per cent. and was made up as follows.

Sodium Chloride .....	11.18 per cent.
Potassium chloride .....	9.56 " "
Monopotassium phosphate .....	13.46 " "
Dipotassium phosphate .....	9.74 " "
Calcium citrate .....	5.78 " "
Dimagnesium phosphate .....	3.94 " "
Magnesium citrate .....	4.26 " "
Dicalcium phosphate .....	7.85 " "
Tricalcium phosphate .....	9.38 " "
Calcium citrate .....	24.85 " "

---

100.00 per cent.

### *Discussion of Results.*

It will be noted from tables I and II that the salts of milk, which are the salts contained in protein-free-milk, do not interfere with the precipitation of sulfates. When amounts of inorganic sulfur were incorporated in samples of a synthetic milk salt mixture, either in the concentration in which it was found to be contained in protein-free-milk or in a much larger concentration (to the extent in which organic sulfur was found to be contained in protein-free-milk), the theoretical amounts of that form of sulfur were quantitatively recovered. The total amounts of sulfur were also quantitatively recovered when allowance is made for the amount contained in the synthetic milk salt mixture itself.

We, therefore, conclude that the salts in milk do not interfere with the direct precipitation of sulfates and that the results secured on the distribution of the various forms of sulfur in protein-free-milk are true.

Table IV shows the distribution of the various forms of sulfur in protein-free-milk from three different breeds of cows. The outstanding point to be gathered from this table is that the percentage of total sulfur is considerably greater than the percentage of total sulfates; also that protein-free-milk contains more total sulfates than inorganic sulfates.

Table V indicates that protein-free-milk, like other biological materials, such as urine, contains at least three different forms of sulfur, namely, inorganic sulfates, ethereal sulfates, and organic sulfur. The significant point brought out by this table is that *organic sulfur represents 66 to 76 per cent. of the total sulfur in protein-free-milk*, as evidenced by results obtained from samples of three different breeds of cows.

The character of these results have an important bearing on nutrition work in connection with the nutritive value of pure isolated proteins. Osborne and Mendel in their early attempts to explore the biological value of pure proteins from different sources were unable to secure any growth when either lactalbumin, edestin, cotton-seed globulin or squash seed globulin served as the only source of muscle building material.<sup>4</sup> When protein-free-milk, however, was incorporated in their rations to serve as the source of salts, previous failure was changed to success in different degrees, depending on the character of protein. Protein-free-milk was not, however, nitrogen free, containing 0.5 to 0.6 per cent. The supplementary value of protein-free-milk could, of course, be explained to some extent on the basis of its content of foreign nitrogen. McCollum and Simmonds suggested the presence of amino-acids in protein-free-milk.<sup>5</sup> From the fact that one of us (Sure) has demonstrated that cystine is a growth-limiting factor both in lactalbumin<sup>1</sup> and edestin,<sup>6</sup> and from the further fact that protein-free-milk contains about three-quarters of its total sulfur in the organic form, the supplementary value of protein-free-milk that Osborne and Mendel obtained with pure proteins could be explained by two possibilities: that protein-free-milk contains either cystine, or an organic sulfur compound that the animal organism can readily transform into cystine.

*Summary.*

1. Protein-free-milk contains three different forms of sulfur, namely, inorganic sulfates, ethereal sulfates and organic sulfur.

2. *Protein-free-milk contains the greater part of its sulfur in the organic form.*

## BIBLIOGRAPHY.

1. Sure, B., *J. Biol. Chem.*, 43, 1920, 457.
2. Folin, O., *J. Biol. Chem.*, 1, 1906, 131.
3. Söldner: *Landw. Versuchs.*, 35, 1883, 361.
4. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 31, 1917, 155.
5. McCollum, E. V., and Simmonds, N., *Am. J. Phys.*, 45., 1918, 284.
6. Sure, B., *Am. J. Phys.*, 61, 1922, 1.





## AMINO-ACIDS IN NUTRITION.

### VI. The Nature of the Supplementary Value of Protein-free-Milk to the Total Proteins of Milk.

By BARNETT SURE.

*From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville.*

In a previous paper<sup>1</sup> the author produced experimental evidence to the effect that cystine is a primary and tyrosine a secondary growth-limiting factor in lactalbumin. Since Osborne and Mendel<sup>2</sup> were able to secure growth using lactalbumin as the source of protein only in the presence of protein-free-milk the suggestion was made that protein-free-milk contains either cystine or organic sulfur that the animal organism may be able readily to transform into cystine; also that protein-free-milk contains traces of the hydroxy-phenyl nucleus of proteins.

In the preceding paper<sup>3</sup> chemical evidence is presented showing that the greater part of the total sulfur of protein-free-milk is present in the organic form.

This paper deals with the role of protein-free-milk as a supplement to the total proteins in milk, when casein and lactalbumin are introduced in the ration in relatively the same proportion as that in which they naturally occur in milk.

The purpose of this investigation was to ascertain two points.

1. Whether lactalbumin has any supplementary value to casein when the total proteins of milk are introduced at a 10 per cent. level.

2. Whether protein-free-milk may serve as a supplement to the total proteins of milk at a level of 10 per cent.; and if so, whether its supplementary value could be explained, at least partially by virtue of its organic sulfur or cystine content, just as in the case of lactalbumin itself.

#### *Preparation of Materials.\**

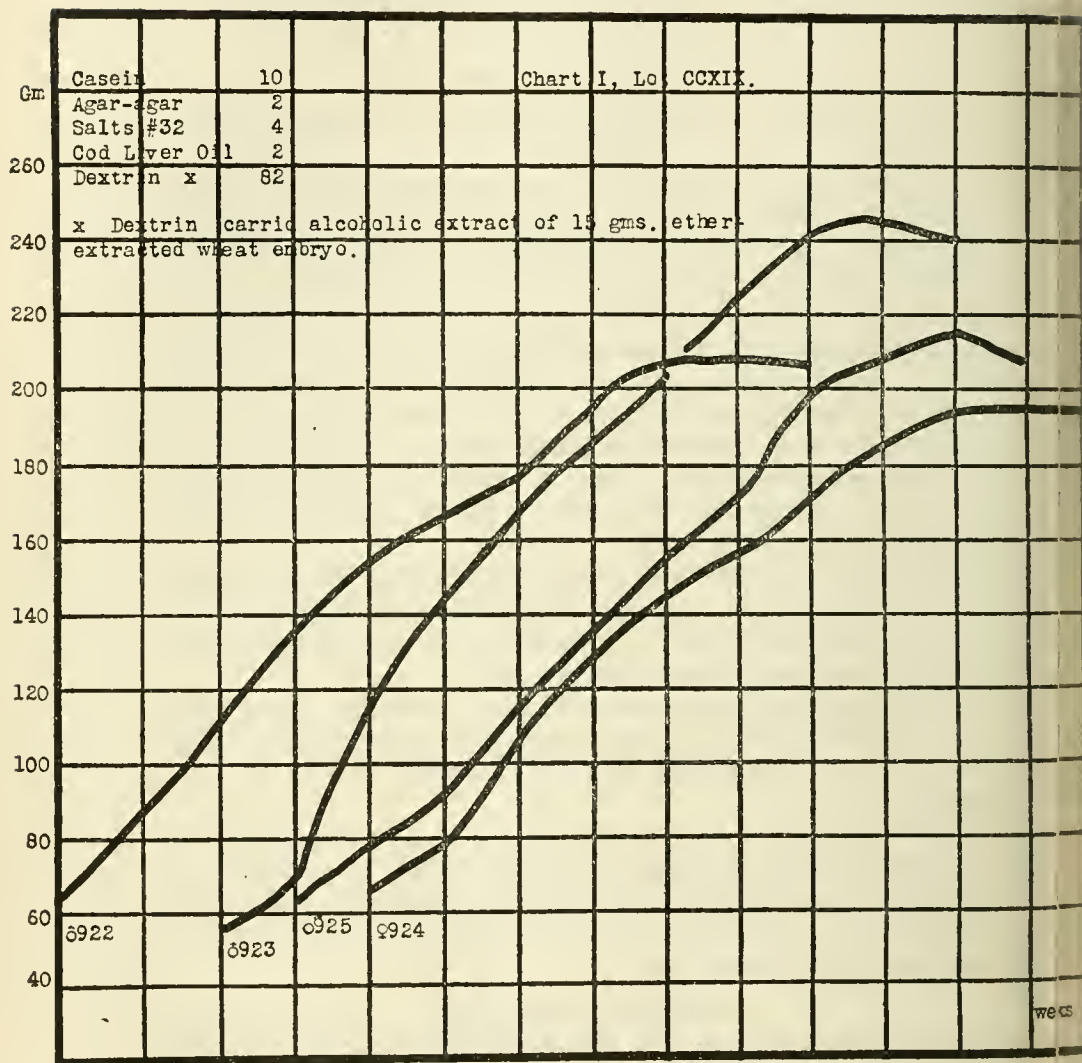
The casein was prepared from skimmed milk in cheese vats, by precipitating with dilute acetic acid, dissolving in alkali, and reprecipitating

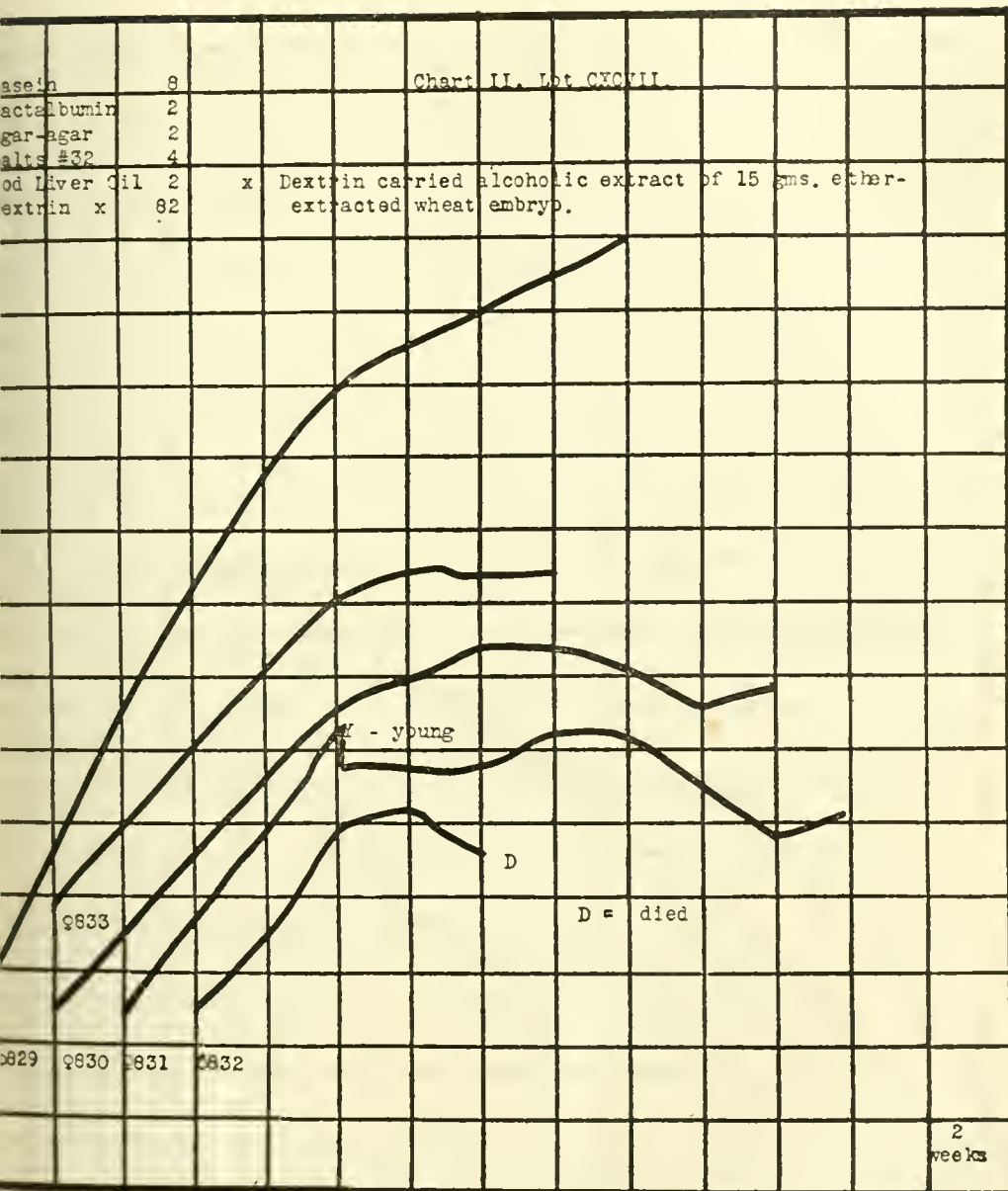
<sup>1</sup> Sure, B.: *J. Biol. Chem.*, 43, 1920, 457.

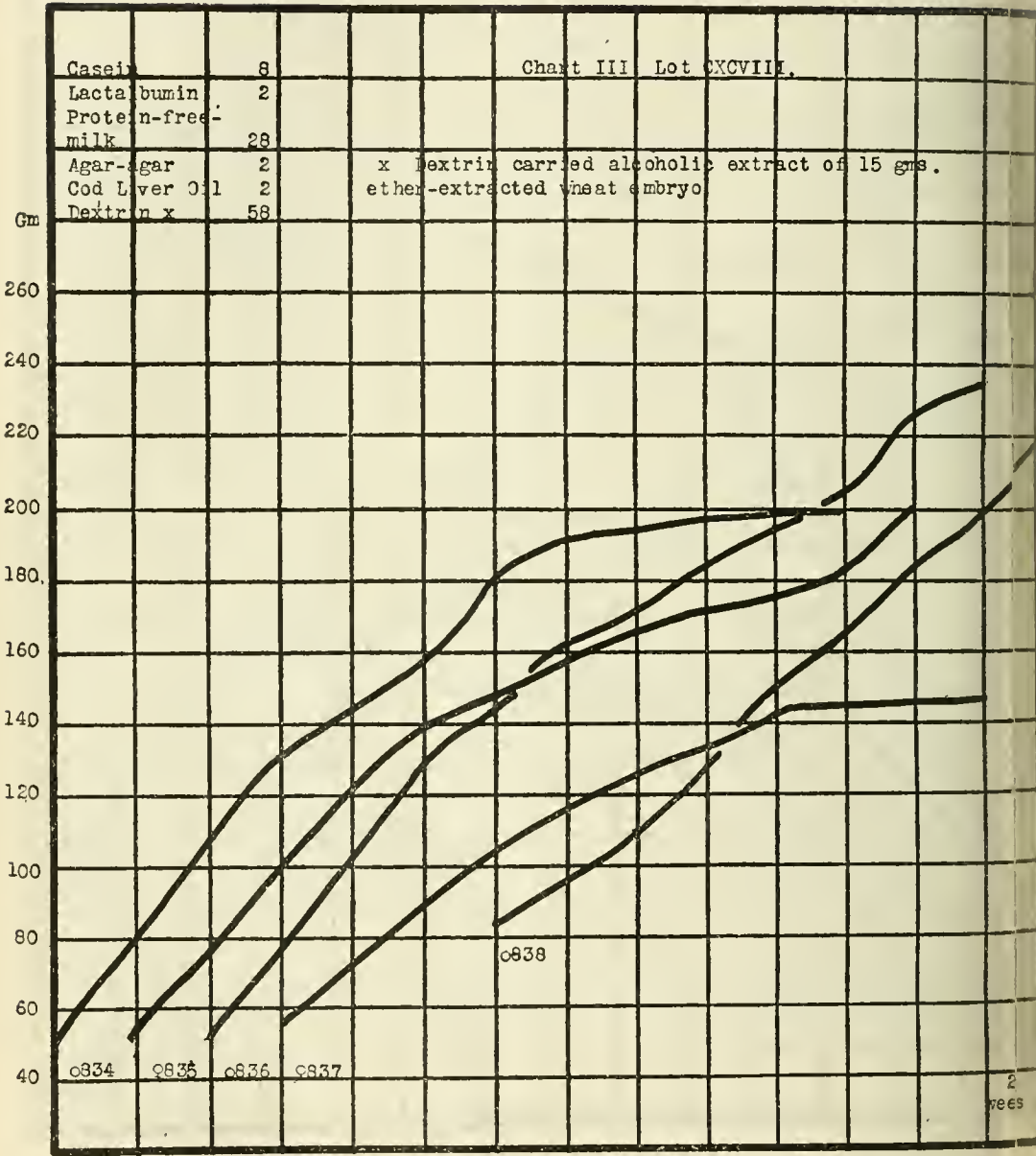
<sup>2</sup> Osborne, T. B., and Mendel, L. B.: *J. Biol. Chem.*, 31, 1917, 155.

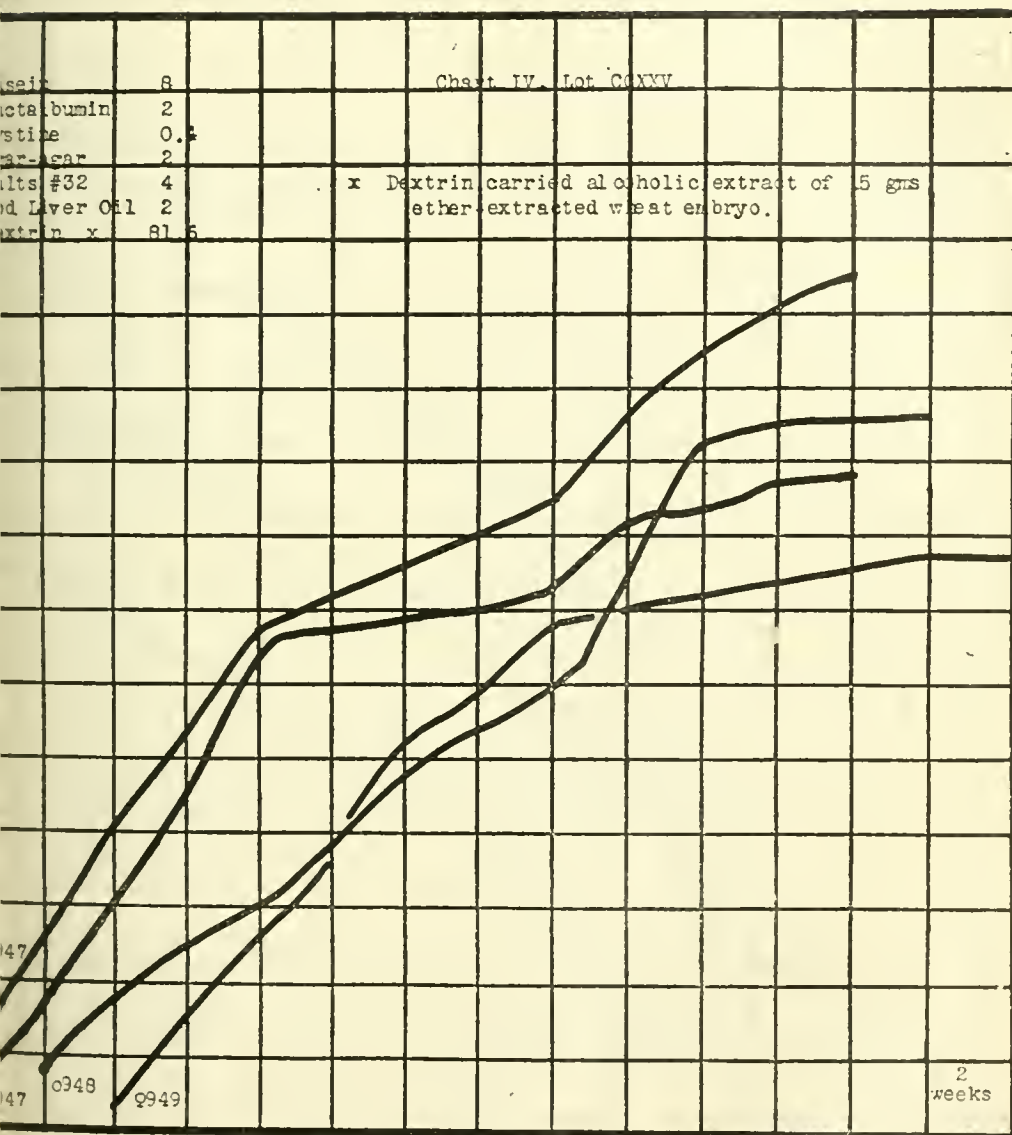
<sup>3</sup> Sure, B.: *J. Metabolic Research*, 3, 1923, 365.

\* The above materials were prepared in the Department of Dairying and Agricultural Chemistry of the University of Wisconsin, Madison, Wis.

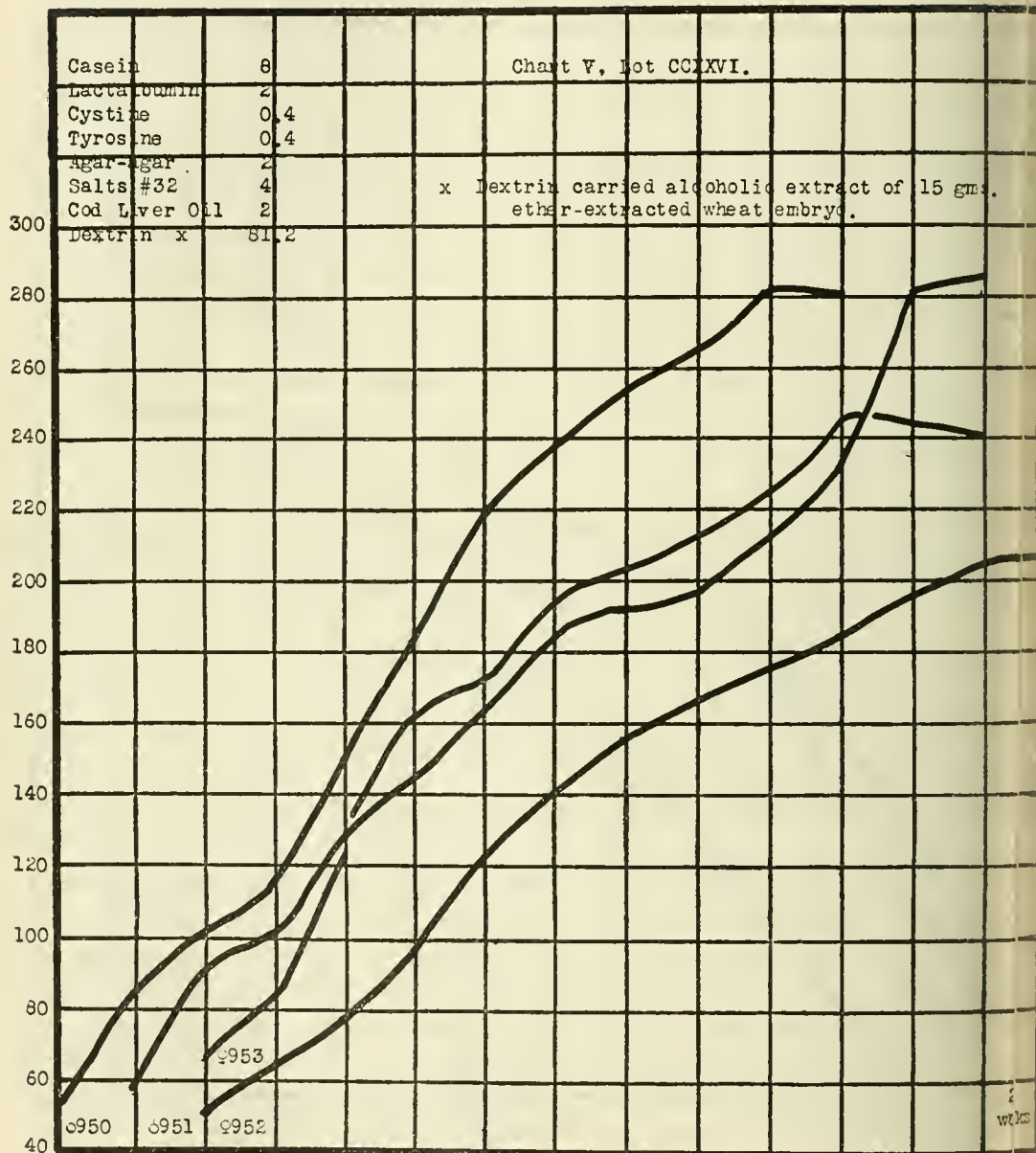












with acid. The same process was continued three times. The casein was then collected from the vats, and after the water had been squeezed out with a press, it was washed for a week in a tub with dilute acetic acid to remove all salts. The protein was then dried at 70 degrees Centigrade in a current of air and finally extracted with hot alcohol.

The lactalbumin was prepared according to a method previously described.<sup>1</sup>

The protein-free-milk was prepared as follows: The lactalbumin whey was heated in galvanized pails to a boil to insure the removal of all traces of albumin. The whey was then filtered and allowed to evaporate on a steam bath to a concentrated solution, and then further dried to a yellowish powder at 70° C. in the presence of a current of air. The material gave a nitrogen content of 0.6 per cent.

The results of the experiments are summarized in the following charts and tables:

Chart I, Lot 219. When casein serves as the only source of protein at a 10 per cent. level a fair amount of growth takes place.

Chart II, Lot 197. This chart clearly indicates that 2 per cent. lactalbumin cannot replace 2 per cent. casein in milk, and that at a 10 per cent. protein level lactalbumin has no supplementary value to the casein in milk. To be sure, one animal made remarkable growth, but four failed. This experiment was repeated with virtually the same results.

Chart III, Lot 198. In this ration 28 per cent. protein-free-milk replaced the 4 per cent. salt mixture and 24 per cent. dextrin. The considerable improvement in growth as compared with the preceding experiment, Lot 197, is quite evident. That protein-free-milk acts as a supplement to the total proteins of milk is unmistakable.

Chart IV, Lot 225. The new factor introduced in this ration is 0.4 per cent. cystine, and it will be noted from the nature of the curves that it replaces very well the 28 per cent. protein-free-milk in the ration as a supplement to the casein and lactalbumin in milk.

Chart V, Lot 226. It is not quite clear from an examination of the curves in this chart and the preceding (Lot 225) whether tyrosine has any significant supplementary role in the total proteins of milk. When the amount of food consumption, however, is taken into consideration, and the gain in grams calculated per gram of protein intake, the tyrosine fed lot seemed to have made about 7 per cent. more growth. (See Table 2.)

### *Discussion.*

When lactalbumin is introduced in the ration in relatively the same proportion to the casein as it is found in milk, it shows no supplementary value to the casein when the total proteins are fed at a 10 per cent. level. Whether lactalbumin has any supplementary value to the casein in milk at higher levels of protein intake is still open for investigation.

Protein-free-milk introduced in the ration to the extent of 28 per cent., replacing the 4 per cent. salt mixture and 24 per cent.

<sup>1</sup> Sure, B.: *J. Biol. Chem.*, 43, 1920, 457.

TABLE I  
Showing Gains in Weight in Grams

Protein	Per Cent	Lot Number	Rat Number	Sex	WEIGHT OF ANIMALS							
					Initial	Four Weeks	Eight Weeks	Twelve Weeks	Sixteen Weeks	Twenty Weeks	Twenty-two Weeks	Twenty-four Weeks
Casein.....	10.0	219	{ 922 923 924 925	♂ ♂ ♀ ♂	65 53 63 64	110 115 107 88	152 171 146 132	177 203 156 171	207 242 187 208	205 237 196 205	... ... ... ...	... ... ... ...
Casein.....	10.0	197	{ 829 830 831	♂ ♀ ♀	55 47 47	106 80 87	164 113 128	217 137 117	234 146 124	259 130 95	... 138 103	... ... ...
Lactalbumin.....	2.0			♂ ♀ ♀	52 77	69 142	99 170	88 173	(D) ...	... ...	... ...	... ...
Casein.....	8.0	198	{ 834 835	♂ ♀	51 51	87 88	142 138	182 159	190 163	200 180	197 204	... ...
Lactalbumin.....	2.0			♂ ♀	51 56	90 81	143 114	172 131	195 142	228 145	233 ...	... ...
Protein-free-milk.....	28.0		{ 837 838	♂ ♂	56 83	110	163	218	236	...	...	...
Casein.....	8.0	225	{ 946 947	♀ ♂	48 55	102 122	173 175	165 178	180 212	209 249	... ...	216 266
Lactalbumin.....	2.0			♂ ♀	57 45	88 96	116 144	145 175	192 172	217 188	... ...	266 233
Cystine.....	0.4		{ 948 949	♂ ♀	...	...	...	...	...	...	...	190
Casein.....	8.0	226	{ 950 951	♂ ♂	53 57	100 100	150 140	220 183	253 195	265 234	... ...	280 286
Lactalbumin.....	2.0			♂ ♀	50 65	79 128	125 171	158 193	177 227	193 235	... 240	204 ...
Tyrosine.....	0.4		{ 952 953	♀ ♀	...	...	...	...	...	...	...	...

D = Died.

D = Died.

dextrin, shows considerable supplementary value to the total proteins of milk, but 0.4 per cent. cystine shows similar biological response; so the nature of the supplementary value of protein-free-milk must be credited to the foreign nitrogen which it carries rather than to the superior value of its salt mixture, and the results of the experiments corroborate the contention made in a previous paper<sup>1</sup> that protein-free-milk carries either cystine, or organic sulfur that the animal organism can readily transform into cystine.

TABLE II  
Showing Gain in Grams Per Gram of Protein Intake

Protein	Per Cent	Lot Number	Gain in Weight	Amount of Protein Consumed	Gain Per Gram of Protein Intake
Casein.....	10.0	219	Grams 598	Grams 576	Grams 1.04
Casein.....	8.0	197	483	600	0.80
Lactalbumin.....	2.0				
Casein.....	8.0	198	723	685	1.05
Lactalbumin.....	2.0				
Protein-free-milk.....	28.0				
Casein.....	8.0	225	700	690	1.01
Lactalbumin.....	2.0				
Cystine.....	0.4				
Casein.....	8.0	226	785	725	1.08
Lactalbumin.....	2.0				
Cystine.....	0.4				
Tyrosine.....	0.4				

Table 2, in addition to indicating that protein-free-milk carries cystine or some similar organic compound which plays a significant role as a supplement to proteins in nutrition, also suggests that tyrosine may serve as a secondary growth-limiting factor. The casein-lactalbumin-protein-free-milk ration (Lot 198) was somewhat better than the casein-lactalbumin-cystine ration (Lot 225); and since the group of animals on the casein-lactalbumin-cystine-tyrosine ration (Lot 226) made 7 per cent. more growth than on the control experiment (Lot 225), it is possible that protein-free-milk is also furnishing traces of tyrosine. Although casein is quite satisfactory for its tyrosine content, it

is quite possible that at an 8 per cent. plane of intake it is not furnishing the optimum amount necessary for growth.

*Summary.*

1. Lactalbumin has no supplementary value to the casein in milk when the total proteins of milk are fed at a 10 per cent. level.
2. Protein-free-milk has a significant supplementary value to the total proteins of milk at a 10 per cent. plane of protein intake which may be attributed either to cystine or to some organic sulfur compound which the animal organism can readily transform into cystine.
3. Protein-free-milk may also have additional supplementary value when introduced to the extent of 28 per cent. of the total ration by adding traces of tyrosine.

## AMINO-ACIDS IN NUTRITION

### VII. Further Studies on the Cause of the Nutritive Inadequacy of the Proteins of the Georgia Velvet Bean (*Stilzlobium Deeringianum*).

By BARNETT SURE

*From the Laboratory of Agriculture Chemistry, University of Arkansas, Fayetteville.*

Recently the author, employing a modified method of studying amino-acid deficiencies in proteins, has produced experimental evidence<sup>1</sup>, showing cystine to be a growth-limiting factor in the proteins of the Georgia velvet bean. Finks and Johns<sup>2</sup> agree with the writer to the extent that the proteins in question are biologically unsound, but disagree as to the cause of their nutritive inadequacy. They ascribe the cause of the poor quality of the proteins in question to indigestibility rather than to specific amino-acid deficiency.

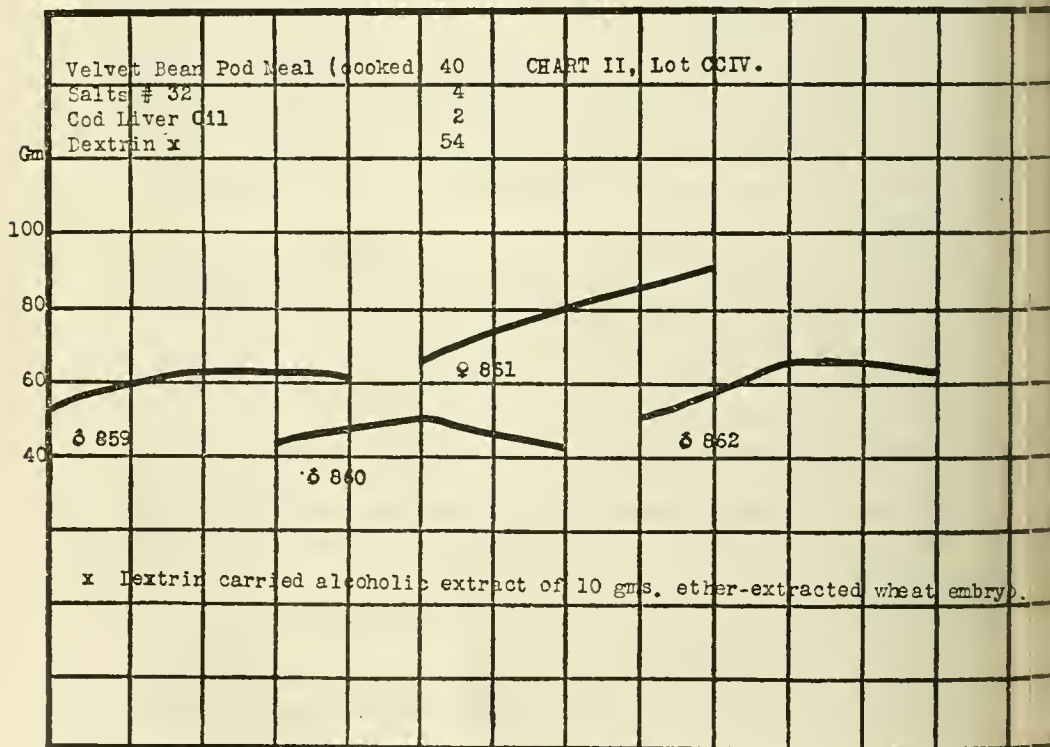
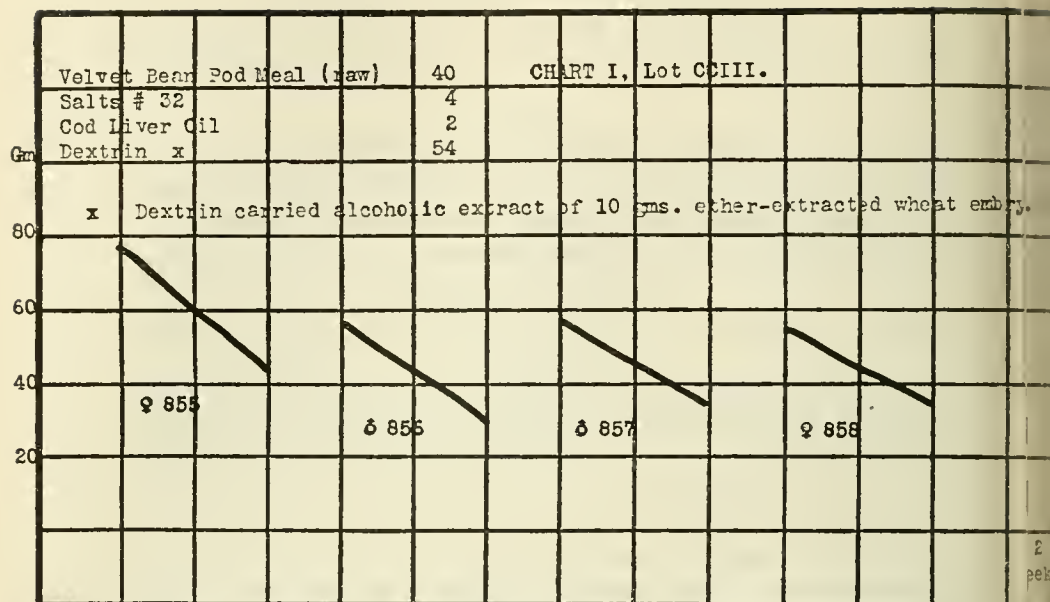
In this paper further experimental data are presented showing that there are at least two factors responsible for the nutritive deficiency of the velvet bean proteins when the pod meal, fed at 8 and 11 per cent of protein intake, serves as the source of muscle building tissue: (1) Indigestibility. (2) Amino-acid Deficiency.

This work deals with the velvet bean pod meal as a source of protein, which, of course, would include all the proteins of the seed and any additional nitrogenous material derived from the pods. Since Finks' and Johns' investigations deal with the isolated globulins from the seed, our work and theirs cannot be considered exactly comparable. It is unfortunate, however, that those investigators employed protein-free-milk, introducing foreign nitrogen in their rations; and it is quite possible, in view of what has been presented in the two preceding papers, that their protein-free-milk was furnishing considerable of the cystine complex.

The results of the experimental data are presented in the following charts and tables:—

Chart 1, Lot 203. This chart indicates that when 40 per cent of the raw Georgia velvet bean pod meal serves as a source of pro-



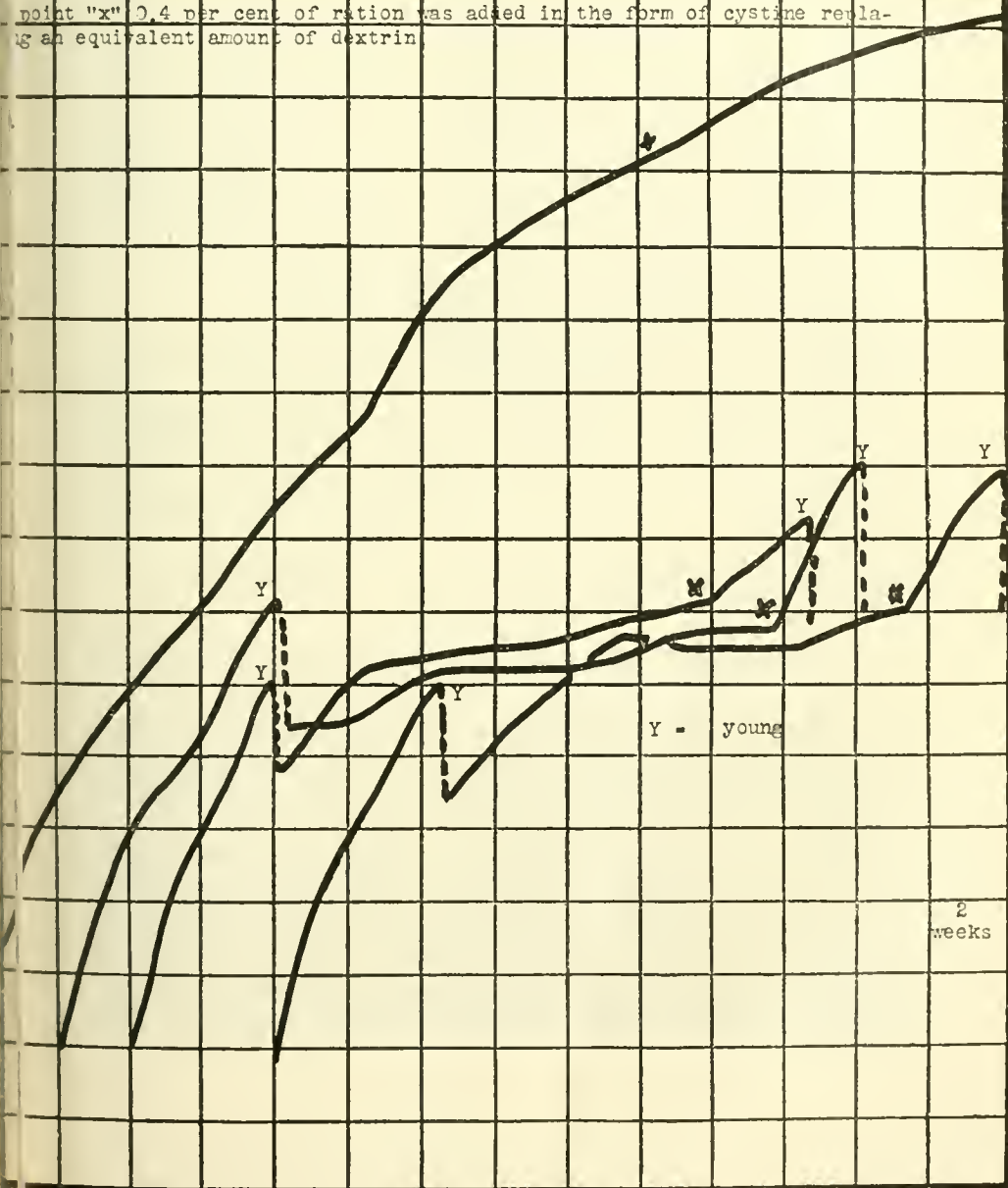


Velvet Bean	Pod Meal	40
Casein		9
Alts #32		4
Pod Liver Oil		2
Dextrin	x	45

Chart III, Lot CCVI.

Dextrin carried alcoholic extract of 10 gms. ether-extracted wheat embryo.

point "x" 0.4 per cent of ration was added in the form of cystine replacing an equivalent amount of dextrin



tein, introducing 8 per cent. of total protein, rapid decline in body weight is observed during the first six weeks of experimentation.

TABLE I  
Showing Gains in Weight in Grams

Protein, Per Cent	Lot Number	Rat Number	Sex	WEIGHT OF ANIMALS	
				Initial	Four Weeks
Velvet Bean, 11..... Gelatin, 9.....	272	1127	♂	57	70
		1128	♀	56	59
		1129	♂	64	65
		1130	♂	57	63
Velvet Bean, 11..... Cystine, 0.4..... Gelatin, 9.....	273	1131	♂	59	83
		1132	♂	60	100
		1133	♂	58	75
		1134	♀	52	75

TABLE II  
Showing Gain in Grams Per Gram of Protein Intake

Protein Per Cent	Lot Number	Gain in Weight	Amount of Protein Consumed	Gain Per Gram of Protein Intake
		Grams	Grams	Grams
Velvet Bean, 11..... Gelatin, 9.....	272	23	94	0.27
Velvet Bean, 11..... Cystine, 0.4..... Gelatin, 9.....	273	104	107.2	0.97

This lot, which had cystine in their ration, made 250% more gain than the preceding lot.

Chart 2, Lot 204. When 40 per cent. of the *autoclaved* velvet bean pod meal is introduced in the ration as a source of protein, fairly good maintenance instead of decline in body weight is obtained.

Chart 3, Lot 206. When 8 per cent. of the total proteins of the pod meal are fortified with 9 per cent casein, excellent growth is secured, and three mothers have all succeeded in partially rearing their young for the first 10 to 19 days of lactation. The young have in each case been reduced to 4 and the mothers have maintained their body weight during the lactation period. At point

"x" 0.4 per cent. of the total ration was added in the form of cystine, replacing an equivalent amount of dextrin. The males and females were separated during the succeeding five weeks, and then rebred. The improvement in the extent of rearing of the young during the second lactation period due to the presence of cystine in the ration is shown in Table III.

TABLE III

Showing the Role of Cystine in Reproduction

During their first lactation period the mothers on ration CCVI had no cystine in their diet. During their second lactation period and five weeks preceding their second breeding 0.4 per cent cystine replaced an equivalent amount of dextrin in the ration.

Gains and loss in weight, given in grams, are indicated by plus (+) and minus (-) signs respectively.

Rat Number	LACTATION PERIOD I (Cystine Absent in Ration)			LACTATION PERIOD II (Cystine Present in Ration)		
	Mother	Young	Total Gain of Mother and Young	Mother	Young	Total Gain of Mother and Young
869	+1	+16	+17	+3	+52	+55
870	+5	+14	+19	+1	+34	+33
871	+3	+15	+18	-4	+37	+33
Total gain in weight of the three mothers during the lactation periods.....			54			123

Experimental evidence is presented that, even after the Georgia velvet bean pod meal is autoclaved for one hour at 15 lbs. pressure (thus rendering the proteins digestible), the proteins therein when fed at 8 per cent. and 11 per cent. planes of intake are biologically inadequate for growth. This is in agreement with the previous findings of Sure and Read on the Biological Analysis of the Seed of the Georgia Velvet Bean,<sup>3</sup> and corroborates the later work of Sure.<sup>1</sup> Autoclaving the pod meal changes the biological response from rapid decline of body weight to maintenance (see Chart 2, Lot 204). This improved condition of the young experimental animals may be ascribed to two factors: (1) destruction of toxicity of the seed, which factor has

been discussed in a previous publication<sup>3</sup>, and (2) improvement in the digestibility of the proteins of the seed. That the coagulated proteins of the Georgia velvet bean have a decidedly higher digestibility coefficient than the unheated proteins is also evident from chemical evidence presented by Waterman and Jones<sup>4</sup>.

That indigestibility is not the only actor responsible for the deficiency of the proteins in question is evident from the following experiments:

An 8 per cent. pod meal protein-cystine-gelatin ration (the pod meal being introduced as 40 per cent. of the ration) showed 50 per cent. more growth than an 8 per cent. pod meal-protein-gelatin ration in the absence of cystine, when fed to young albino rats for a period of two months. The protein content of velvet bean meal (seed without hulls) has been found to be 27.5 per cent., while the protein content of the velvet bean pod meal has been found to be only 20.0 per cent.; therefore, in order to obtain results comparable with those of previous work,<sup>1</sup> 55 per cent. of the pod meal instead of 40 per cent. had to be used as a source of protein. Tables I and II clearly show that during the first 30 days, which is the period of most rapid growth, the lot on the pod meal cystine-gelatin ration made 250 per cent. more growth than the lot receiving the pod-meal-gelatin ration without cystine. The velvet bean pod meal of that ration was autoclaved for one hour at 15 lbs. pressure, so the digestibility factor was entirely eliminated.

The addition of 9 per cent. casein to 8 per cent. autoclaved velvet bean pod meal proteins shows not only excellent growth but partial success in reproduction. (See Chart 3 and Table III). Although Osborne and Mendel have obtained responses to cystine when casein was fed at levels below 12 per cent. of protein intake, the author cannot help but conclude that the 9 per cent. casein in ration 206 is supplying enough cystine to supplement the inadequate amount contained in the velvet bean pod meal in so far as *growth* is concerned. That there is a response to cystine to this pod meal protein-casein ration from the standpoint of *reproduction* became evident from the following experiments:

In connection with further studies on the supplementary role of lactalbumin to casein in milk at higher levels of protein intake reproduction was tried on the following rations:



<i>Ration No. 274</i>		<i>Ration No. 275</i>	
Casein	20	Casein	16
Agar-agar	2	Lactalbumin	4
Salts No. 32	4	Agar-agar	2
Cod Liver Oil	2	Salts No. 32	4
Dextrin*	72	Cod Liver Oil	2
		Dextrin*	72

\* Dextrin carried alcoholic extract of 15 gms. ether-extracted wheat embryo as source of water-soluble B vitamin.

Of two females bred on ration 274, only one became pregnant, mother No. 1139. She gave birth to two young weighing 8 grams. On the third day after birth they both weighed 9 grams and were disposed of by the mother on the following day. Of two females also bred on ration 275, one became pregnant, mother No. 1143. She gave birth to 4 young weighing 21 grams, which on the third day weighed 23 grams. These young were disposed of by the mother on the fifth day.

From the above experiments it is evident that casein, the main protein of milk, even at as high a plane of intake as 20 per cent., is entirely inadequate for reproduction.

That all the proteins of milk, introduced to the extent of 17.5 per cent. of the total ration, are entirely inadequate for reproduction, using the albino rat as the experimental animal, has become evident when breeding was attempted on the following ration:

<i>Ration No. 239</i>	
Skimmed Milk Powder	50 (35 per cent. protein)
Cystine	0.4
Agar-agar	2
Fe Citrate	0.1
Cod Liver Oil	2
Dextrin*	45.5

\*Dextrin carried alcoholic extract of 30 gms. of ether-extracted wheat embryo. During the breeding period 0.4 lysine replaced an equivalent amount of dextrin.

Even in the presence of abundant amounts of vitamins A and B, and the presence of cystine and lysine, in this case three females, Nos. 1004, 1006 and 1007, after ten weeks breeding, did not even become pregnant when the total proteins of milk were employed to the extent of 17.5 per cent. of the total ration. It is evident, then, that cystine even in the presence of lysine is not supplementing the milk proteins, and for that matter is not supple-



menting casein (since casein is present in the milk proteins in the largest proportion) from the standpoint of reproduction.

Table III shows that on an 8 per cent. pod meal protein-9-casein ration considerably better rearing of the young is secured than on either rations, Nos. 274 or 275, where 20 and 16 per cent. casein respectively served as the source of protein. Such results would lead us to conclude that, while casein is supplementing amino-acids to the proteins of the velvet bean from the standpoint of growth, the amino-acids contained in the legume are supplementing the milk protein from the standpoint of reproduction.

That cystine is a limiting amino-acid in both velvet bean protein and casein became a little more clear after cystine was added to ration 206, and the mothers allowed to partake of a velvet-bean protein-casein-cystine diet five weeks previous to re-breeding and also during their second lactation period. Food consumption records show that there was no greater intake of food on part of the mothers during the second lactation period, when they had access to cystine than during their first lactation period when cystine was absent in the ration. Table III clearly indicates that reproduction was much more successful during the second lactation period when cystine was administered in the diet. The degree of success was measured by the total gain in weight of the young during the rearing period, considering at the same time the gain or loss in weight of the mothers.

It is not claimed, after the digestibility factor is dispensed with by autoclaving the velvet bean pod meal proteins, that cystine then shows itself up to be the primary and only growth-limiting factor responsible for the poor quality of the proteins in question. From the character of the relatively small increments of growth secured compared with normal growth, it is quite apparent that such is not the case; but the evidence submitted is comparative with regard to biological responses obtained in the presence of that amino-acid in connection with growth and remarkable responses were secured in that connection.

Recently McCollum, Simmonds and Parsons<sup>5</sup> have improved the technique of the biological evaluation of proteins. In addition to the criterion of growth, degree of success in reproduction and ability to preserve youthful characters have been demonstrated to be factors we must contend with before final judgment is passed on the quality of proteins from various sources. If the degree of success in reproduction depends to a large extent on the pro-

tein moiety, and the synthetic capacity of the mammary gland is limited with respect to certain indispensable amino-acids, then it follows that fecundity, infant mortality, and degree of success in rearing of young must depend in a large measure on the presence of specific amino-acids in the diet in large enough concentration during the lactation period. It is quite possible that amino-acid deficiencies in proteins which cannot be detected from the standpoint of growth, can be detected from the standpoint of reproduction. It cannot be expected, however, that if the deficient quality of a group of proteins in so far as reproduction is concerned is due to a number of amino-acids of different structure, that the addition of any one of these building blocks to the ration is going to change absolute failure in reproduction to complete success. All that can be expected is small but definite differences in the degree of success of rearing of the young, and such evidence has been secured with regard to the role that cystine plays in reproduction in connection with the velvet bean pod meal proteins. Bearing such considerations in mind, and judging from the responses secured due to the incorporation of cystine in the velvet bean protein rations, both from the standpoint of growth and reproduction, it is concluded that, in addition to indigestibility, amino-acid deficiency should be considered as another factor responsible for the nutritive inadequacy of the proteins in question, and that *cystine* is one of the amino-acids responsible for that deficiency.

### *Bibliography*

1. Sure, B.; *J. Biol. Chem.*, 1, 1922, 103.
2. Finks, A. J., and Johns, C. O.; *Am. J. Physiol.*, 57, 1921, 5.
3. Sure, B., and Read, J. W.; *J. Agr. Res.*, 22, 1921, 5.
4. Waterman, H. C., and Jones, D. B.; *J. Biol. Chem.*, 47, 1921, 285.
5. McCollum, E. V., Simmonds, N., and Parsons, H. T.; *J. Biol. Chem.*, 1921, 111-247.

## 17TH FRENCH CONGRESS OF MEDICINE.

*The 17th French Congress of Medicine will meet at Bordeaux,  
Sept. 27 to 29, 1923.*

### PROGRAM.

1. Remote effects of malaria. Speakers: Professor Le Dantec, of the Bordeaux Faculty, with the collaboration of Dr. Hesnard, Naval Physician, Professor of the Bordeaux School of Naval Hygiene, who will deal especially with post-malarial psychoses, and of Dr. Marcel Léger, médecin-major of Colonial troops, Director of the Dakar Physiological Institute, who will deal with visceral lesions of malaria.

A paper will also be presented by Dr. Broden, Director of the State School of Tropical Medicine at Brussels.

2. Relation between the sympathetic and the endocrine glands in pathology. Speakers: Professor Pachon, of the Medical Faculty of Bordeaux; agrégé Professor Perrin, of the Medical Faculty of Nancy.

3. Treatment of meningococcal infections. Speakers: Dr. Dopter, Professor at Val-de-Grace, and Dr. Boidin, Physician of the Paris hospitals. Dr. Dopter will speak particularly of serotherapy, and Dr. Boidin of vaccine therapy.

## STUDIES OF THE VITAMINE POTENCY OF COD LIVER OILS.

### II. The Vitamine Potency of "Spring" Cod Liver Oil.

By ARTHUR D. HOLMES.

*Research Laboratories, The E. L. Patch Company, Boston, Mass.*

In studying the vitamine content of cod liver oil, Osborne and Mendel<sup>1</sup> found that albino rats grew fully as fast when cod liver oil constituted 6 per cent. of their diet as when the diet contained 18 per cent. of dairy butter. Later McCollum<sup>2</sup> showed that cod liver oil was about twenty times richer than butter in vitamine A and recent investigations by Zilva<sup>3</sup> indicate that cod liver oil is two or three hundred times richer than average butter which is the next richest material in fat-soluble A.

From a therapeutic standpoint the physician desires definite information concerning the presence and amount of vitamine A in the particular cod liver oil which he wishes to use, the same as he desires definite information concerning the strength of Ergot, Digitalis, Pituitary and similar medicinal preparations. It was for the purpose of securing data of this sort that investigations on vitamines now being conducted in this laboratory were inaugurated. Among those in progress is one started several months ago for the purpose of securing data relative to possible variation in the vitamine A potency of cod liver oils obtained from fish taken at different seasons of the year and the present paper reports the results of a group of experiments with oils which are being obtained from fish taken in the same locality at more or less frequent intervals during the entire period that cod fish are to be found in this section.

In order to provide cod liver oil of known origin the author obtained at Rockport, Mass., on the 3rd of March, a supply of livers from cod fish caught just off shore a few hours previous. These livers were rendered during the early evening, or within

---

<sup>1</sup> *Jour. Biol. Chem.*, 17, No. 3 (1914), p. 401.

<sup>2</sup> *Jour. Biol. Chem.*, 50 No. 1 (1922), p. 5.

<sup>3</sup> *Biochem. Jour.*, 40 No. 5 (1921), p. 655. *Lancet*, 1, No. 7 (1921), p. 323.

five or six hours after being removed from the fish. A household meat chopper was used to mince the connective tissues so that the oil would separate as fast as it melted. The finely cut livers were heated in the upper portion of a double boiler for half an hour at 90° C. and the oil was filtered from the liver residue with a filter of three thicknesses of cheese cloth. However, some of the finer particles of liver residue passed through the cloth filter and after the oil had stood long enough to allow the water and liver particles to settle, it was decanted and filtered through ordinary laboratory filter paper.

It has been shown by Kennedy and Dutcher<sup>1</sup> that the amount of vitamine A present in milk is entirely dependent upon the nature of the diet of the cow. This conclusion is in accord with the results of the experiments of Hopkins.<sup>2</sup> Drummond<sup>3</sup> et al have reported experiments which furnish very definite evidence to the effect that the vitamine A content of the body fat of hogs is greatly influenced by the nature of the diet of hogs during the growing period. It therefore appears of interest to note here that except for a few fish which contained pieces of bait, the alimentary tracts of the cod fish taken early in March were free from any food, which was in marked contrast to the situation later in the season. The physical appearance of the fish as they were caught confirmed the conclusion that at this particular time the fish were being maintained largely, if not wholly, on their body reserve.

In the commercial preparation of medicinal cod liver oil, it is customary to remove cod liver stearin from crude cod liver oil, but for the purpose of this series of experiments, it was felt that it was desirable to study the vitamine potency of the crude oils since in the "cold press" process it would be extremely difficult to maintain sufficiently uniform operating conditions to insure the removal of uniform amounts of stearin at different seasons of the year, a necessary condition in order to produce uniform samples of pressed oil.

To facilitate identifying the nature of this oil and to make possible a comparison of it with oils studied by other investigators, a determination of the chemical and physical characteristics was made with the following results:

<sup>1</sup> *Jour. Biol. Chem.*, 1, No. 2 (1922), p. 339.

<sup>2</sup> *Biochem. Jour.*, 14, No. 6 (1920), p. 721.

<sup>3</sup> *Biochem. Jour.*, 14, No. 6 (1920), p. 742.



*Chemical and Physical Characteristics of Cod Liver Oil From  
Fish Taken March 3, 1922.*

Specific Gravity at 25° C. ....	0.9206
Refractive Index at 20° C. ....	1.4783
Saponification value .....	191.
Iodine value .....	143.4
Acid value .....	0.6223
Cold Test (clouding points).....	6° C.

The acid value 0.6223 reported above is the lowest acid value obtained for any of the cod liver oils thus far studied, and it will be of interest to compare this acid value with that of oils secured at other seasons of the year. Colorimetric analysis<sup>1</sup> of this sample of early spring cod liver oil showed its color to be 35 yellow and 5 red, which indicates that it was not as dark an oil as the commercial crude cod liver oil previously reported<sup>2</sup> that was found to be 35 yellow and 12 red.

The experimental animals; the constituents and the composition of the experimental diet; and the method of administration of uniform daily doses of cod liver oil employed in this investigation are similar to those discussed in detail in an earlier paper.<sup>3</sup> Data concerning the lighting conditions of our colony room have also been discussed in a previous communication.<sup>4</sup> The accompanying charts supply detailed data concerning the amount of food ingested and the changes in the body weight of the experimental animals during the experimental period.

All the animals were placed on the experimental diet on the same date and the individual weights at the beginning of the experimental period ranged from 59 grams for rat No. 32 to 70 grams for rat No. 28. It appears however, that the body reserve of vitamine A was not the same for all the animals, as rats No. 28 and No. 29 stopped growing from ten to twenty days earlier than the other three animals. At the time when cod liver oil was added to the experimental diet, all the experimental animals were emaciated, and had a rather unsteady gait. All had more or less ophthalmia, but that of rat No. 28 was the most severe.

The amount of special cod liver oil ingested daily varied from 0.00202 grams to 0.01010 grams per animal daily. But a com-

<sup>1</sup> The author is indebted to Dr. David Wesson for his kindness in supplying data concerning the color value of these oils.

<sup>2</sup> *Jour. Metabolic Research*, 2, No. 1 (July, 1922), p. 113.

<sup>3</sup> *Jour. Metabolic Research*, 2, No. 3 (Sept., 1922), p. 361.

<sup>4</sup> *Jour. Metabolic Research*, 2, No. 1 (July, 1922), p. 113.



Chart 4

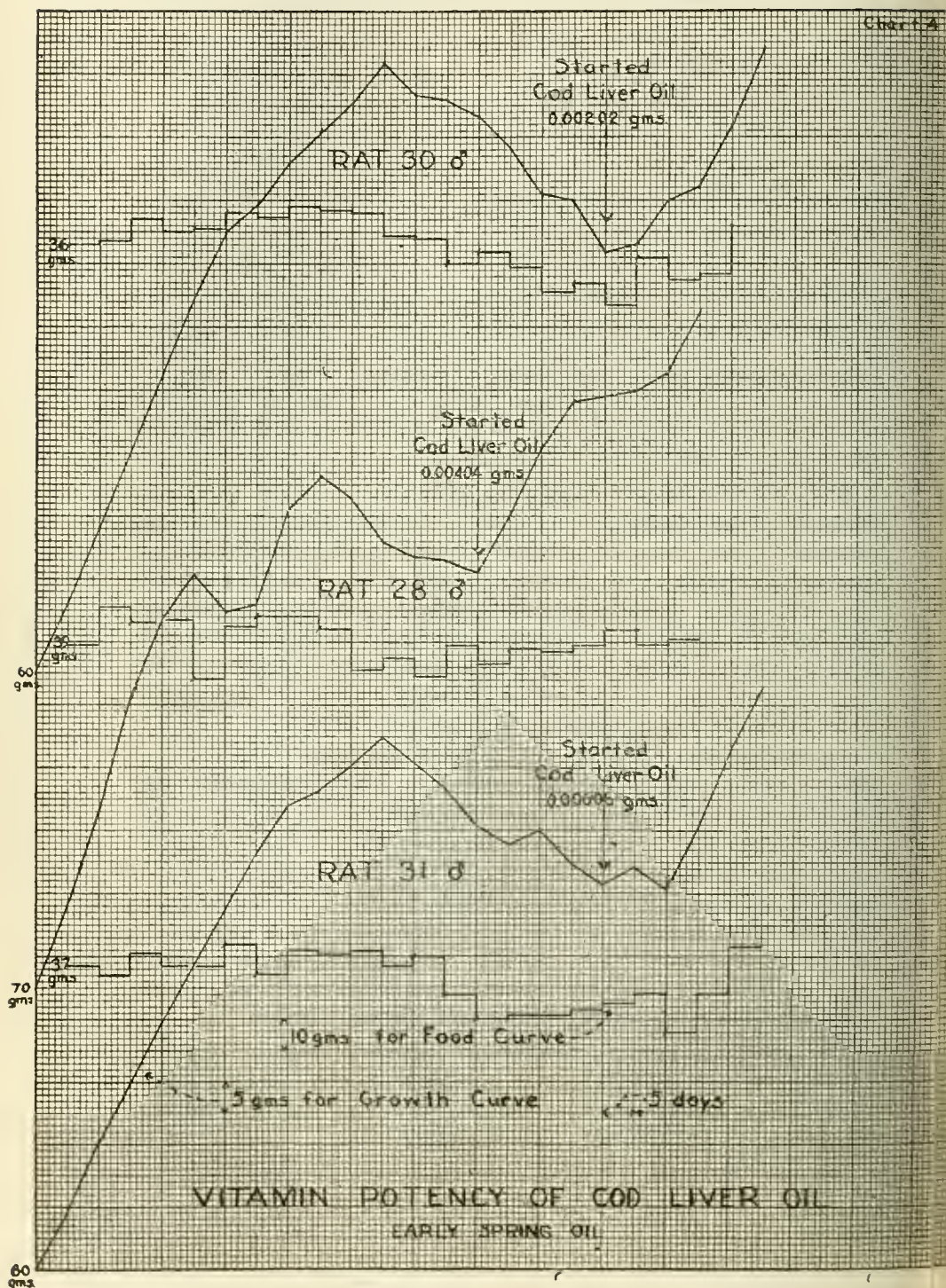
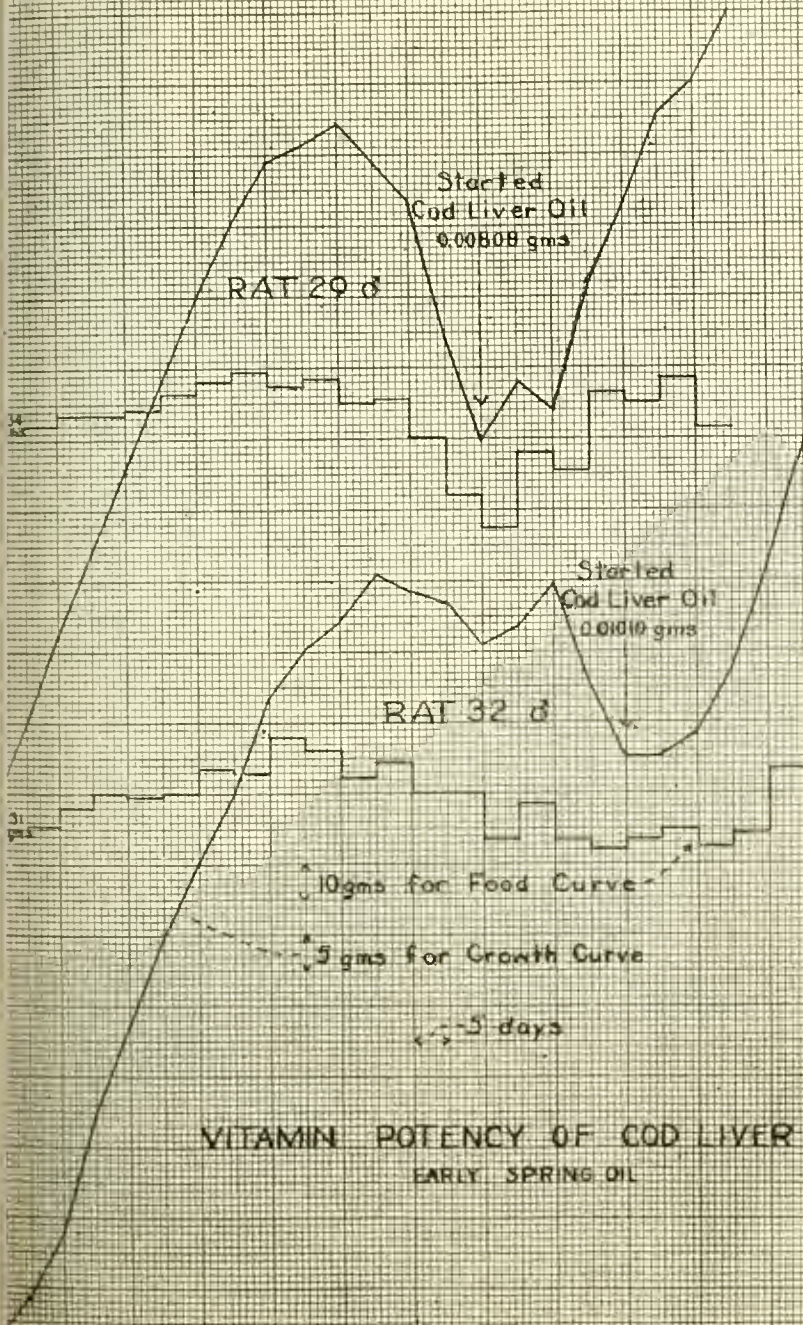




Chart 5



VITAMIN POTENCY OF COD LIVER OIL  
EARLY SPRING OIL

parison of the weight curves of all the rats shows that the rate of body increment is extremely close and one naturally concludes that 0.00202 grams of this particular cod liver oil is as efficacious as 0.01010 grams for correcting this type of malnutrition. These results indicate that cod liver oils from emaciated fish may be highly potent in vitamine A, but the data at present available show that cod liver oils obtained from plump cod fish have a higher vitamine potency than that obtained from emaciated fish.

## THE AFTER EFFECTS OF PROLONGED FASTING ON THE BASAL METABOLIC RATE.

By MARGARETE M. KUNDE.\*

(From the Hull Physiological Laboratory of the University of Chicago)

The work here reported was undertaken at the direction of Dr. Carlson with the view of securing some measurable and objective data on the after effects of prolonged fasting.

There are a number of well controlled experiments, on man and animals, on the effects of prolonged fasting on metabolism during the fasting period,<sup>1</sup> but no one seems to have investigated the possible after effects of fasting on the basal metabolic rate. We have extensive data on the macro- and microscopic<sup>2</sup> changes induced in the various organs by fasting. We have reports of apparent improvement in mental conditions in man as the result of fasting. The tonus<sup>3</sup> of the stomach increases during fasting. Extensive experiments on some invertebrate groups by Child<sup>4</sup> and his co-workers<sup>5</sup> seem to point to a process of rejuvenescence of tissue by starvation. In diabetes in man it appears that partial starvation, in some cases, increases the capacity of the body to utilize sugar.<sup>6</sup>

Since it is established that the young individual has a higher level of oxidative activity than the adult<sup>7</sup>, it would seem to follow that if prolonged fasting actually leads to rejuvenescence or extensive renewal of body cells over that which takes place under ordinary conditions of feeding, such rejuvenescence would be evidenced by an increase in the basal metabolic rate.

It is the purpose of this experiment to determine whether measurable changes occur in the basal metabolic rate as a result of prolonged fasting followed by normal feeding. It seems that this can be done by determining the level of the basal metabolism of the subjects before, during, and after prolonged fasting, keeping the diet, body weight, and mode of living before and after the fast as nearly identical as possible.

### *Experimental Methods.*

The basal metabolism, computed from the oxygen consumption, was used as an index of the oxidative activity of the body

\* National Research Council Fellow in Physiology.



when at rest. For this purpose a Benedict portable respiratory apparatus,<sup>8</sup> with suitable modifications adapted to our needs, was used. Two people and three dogs served as subjects.

The pre-fasting period constitutes the control period. During this period, data on the several subjects establishes the average normal levels of the basal metabolic rate, body temperature, pulse rate, respiration rate, and body weight.

There was no attempt made to regulate the quantity or kind of food eaten by the two persons who served as subjects, other than that one of them, M. K., lived in one of the University Residence Halls for women where there is some dietary supervision, so that it is possible for the occupants of this hall to receive wholesome, well-cooked food approximately balanced in the composition of the various foodstuffs, as estimated by the supervising dietitian. This subject was engaged 12-16 hours daily in graduate studies and in teaching. The other person, F. H., was a worker in a small flour mill. The food which he ate was prepared by himself, and since he has a fairly good knowledge of dietetics and nutrition, he was able to make rather accurate estimations of the daily food requirements for a working man in his capacity. In all cases 12 hours elapsed from the time that food was eaten until metabolism tests were made on the two persons in the morning.

The dogs were fed on an accurately weighed maintenance diet. During the control period this consisted of 250 g. of hashed, lean beef, 100 g. of bread and 250 cc. of whole milk. Cooked bones were fed them from time to time to supply necessary roughage. The dogs were fed once a day, 18 hours before their basal metabolic rates were estimated.

No marked changes occurred in the room temperature in which the tests were made, in spite of the fact that the determinations on M. K. were made daily for a year, and on the other subjects through the changing seasons. The most favorable temperature proved to be between 24.5°-25.5°C. The relatively constant temperature in the metabolism room during the time that tests were made was maintained through several factors. (1) The small room is provided with double doors. (2) It is equipped with an automatic thermostat which guards against over-heating during the winter. (3) The ventilation is such that outdoor air can be admitted equally efficiently through either a small or a large aperture. (4) The metabolism determinations were completed during the summer months by 7 A. M.; during the winter, by 9 A. M. The utilization

of the early morning hours prevented the excessive summer heat from interfering with the experiments. Then, too, the dogs are more easily managed at this time than later in the day, and these regular hours exclude errors due to diurnal variations.<sup>9</sup>

The effect of muscular activity on the metabolism was controlled by having each person relaxed and resting quietly on a couch for at least 30 minutes immediately preceding the test. The dogs were trained to lie quietly on a comfortable pad for at least 45 minutes immediately preceding the determinations and to remain so during the time that they were connected to the respiratory apparatus.

A simple device for connecting the dogs to the Benedict portable respiratory apparatus was perfected. This consists of a rubber dam tightly stretched over one end of a muzzle that serves the same purpose in animal calorimetry that the face mask serves in making determinations of the respiratory exchange of people. The other end of the muzzle is equipped with a metal tube, and a short rubber tubing connects this with the three way valve of the respiratory apparatus. A circumscribed area between the dog's eyes and the corners of the mouth must be cleanly shaven. The dog's nose is gently inserted into the perforation in the rubber dam so that the shaved area makes an air tight approximation with the perforation in the rubber dam. The dogs, when properly trained, offer no resistance to the muzzle. The muzzle is not adjusted until 3 or 4 minutes before the experiment begins. The corner of the mouth must be well inside of the muzzle and all wrinkles carefully smoothed out. The rubber must be pulled far forward under the chin, so that it fits smoothly over the *spina mentalis*.

Tests for tightness with a dog connected to the Benedict portable respiratory apparatus are made by making a duplicate determination of the oxygen consumed and placing a small weight on top of the spirometer bell during one of the determinations. If the muzzle is not tight the determination made with the weight on the spirometer bell will be much higher. Tests for tightness made in this manner check to within 1 or 2%.

The dogs were not confined to a cage but each lived in a roomy, separate booth with ample space for them to walk about freely. Further precaution to prevent the loss of muscle tonus due to laboratory confinement<sup>10</sup> consisted in vigorously exercising the dogs daily either by taking them out of doors or by allowing them



to play and run about freely two or three hours in a large attic over the laboratory. The dogs had been living in the laboratory from six weeks to four months before experimentation on them began.

*The Fasting Period* as here used designates the time during which the subjects completely abstained from all foods. They were allowed to drink water *ad libitum*. During the fasting period the two persons continued their daily occupations, as mentioned above. The pulse, temperature and body weight were taken immediately after, and the respiration rate during the determination of the metabolism. Each of the two persons fasted for 15 days. Dog No. 1 fasted for 37 days, dog No. 2, for 40 days, dog No. 3, for 41 days.

*The Period After Fasting.* The duration of this period varies with the several subjects, but in each case the term is used to indicate the time from the breaking of the fast until the end of the experiment under discussion. For M. K. the length of this period was six months; for F. H.  $9\frac{1}{2}$  weeks. For Dog No. 1 this period extended through  $9\frac{1}{2}$  months; Dog No. 2 for  $4\frac{1}{2}$  months; Dog No. 3 for  $3\frac{1}{4}$  months. The predominant motive during this period was to keep the experimental conditions exactly the same as during the control period. Dogs No. 1 and No. 2 were not fed on the maintenance diet for the first four days of this period because of their inability to retain food. After the first four days each of these dogs received the maintenance diet until the respective body weights of the control period was reached. Then the food was reduced in amount in an attempt to keep the body weight from increasing over that of the control period. The experimental work on Dog No. 3 was interrupted for the first ten days of this period. After that this dog received the maintenance diet until the body weight of the control period was reached.

#### *Methods of Calculation.*

All calculations for determining the number of calories of heat produced for 24 hours are based on the amount of oxygen consumed during the experimental period and assuming that the respiratory quotient was .82. Since  $\text{CO}_2$  excretions were not estimated it was impossible to determine the actual respiratory quotient for the several subjects. But it has been shown that the respiratory quotient of normal individuals, when in the postab-

sorptive condition, is fairly constant or at most varies only 2 or 3 points from the quotient<sup>11</sup> 0.82, i. e. when the diet is fairly uniform and consists of a mixture of the foodstuffs. It is also known that the caloric value of oxygen varies only 8% from the greatest possible extremes of the respiratory quotient<sup>12</sup> (1.—0.70). If one arbitrarily chooses a respiratory quotient midway between the two extremes, the greatest possible error that could be introduced is about 4%. But since the respiratory quotient normally varies only 2 or 3 points from the quotient 0.82, the error introduced into the calculations by arbitrarily using 0.82 can only be 1 or 2%. This error is far less than would be introduced in attempting to determine the amount of CO<sub>2</sub> excreted during the test with the Benedict portable respiratory apparatus, owing to the great difficulty in accurately weighing the cumbersome soda lime containers and the possibility of excreting more CO<sub>2</sub> than is actually produced in the short period through excessive ventilation of the lungs.

The experimental period for each person was about 10 minutes; for each dog 15 minutes. The graphic method was used to determine the number of cc. of oxygen actually consumed during each period. Care was taken to begin and end the period at the end of a normal respiration. A true base line was traced on the drum by means of a signal magnet during the time that the experiment was in progress. Greater accuracy in measuring the results can be obtained by using this line rather than the edge of the paper. The motor was set in motion 4-5 minutes before the experiment actually began. This assures the complete removal of all of the CO<sub>2</sub> in the circuit and often eliminates wide temperature changes in the spirometer. After the subject is disconnected the motor is allowed to run until the writing point traces a straight line. This point indicates the complete removal of all of the CO<sub>2</sub>. The amount of O<sub>2</sub> consumed is indicated, not by the end of the last normal respiration of the subject but at the level where the writing point began to trace a straight line. The paper is removed from the drum and fixed with shellac, laid on a smooth table and the cc. of O<sub>2</sub> consumed measured by means of the spirometer scale according to the scheme which explains Fig. 3.

The distance R B — V B on the measuring scale indicates the actual number of cc. of O<sub>2</sub> consumed by the subject during the period.

The Benedict method, slightly modified and greatly simplified,

was used to convert the cc. of  $O_2$  into calories of heat as follows: If the distance R B — V B equals 2100 cc. of  $O_2$  and the experimental period 10 minutes and 6 seconds; the B. P. 74.1; the temperature of the spirometer at the beginning of the determination  $24^{\circ}.5\text{ C}$ ; the temperature at the end of the experiment  $25^{\circ}\text{ C}$ ; the increase in temperature in the spirometer is  $.5^{\circ}\text{ C}$ . The average temperature in the spirometer during the experiment is  $25^{\circ}\text{ C}$ . Since an increase of temperature of  $1^{\circ}\text{ C}$ . equals  $1^{\circ}.8$  cc.  $O_2$  per minute (Benedict calibration factor), an increase of  $.5^{\circ}\text{ C}$  would equal .9 cc. of  $O_2$  per minute.  $.9 \times 10.1$  equals 9.9 or 10 cc. of  $O_2$ . 2100 cc. of  $O_2 + 10$  cc. equals 2110 cc. of  $O_2$  consumed during the period, corrected for temperature changes in the spirometer. The caloric value of liter of  $O_2$  when the respiratory quotient is .82 equals 4,825.

Log. of 2110 .....	.32428
Log. factor for reducing $25^{\circ}\text{ C}$ and 74.1 mm. B. P. to standard conditions .....	.95088
Log. of $60 \times 24 \times 4.825$ equals .....	.84186
	<hr/>
	.11702
Subtracting Log. of 10.1 .....	.00432
	<hr/>
	.11270
Anti log. of .11270 .....	1296

The total number of calories of heat produced for 24 hours equals 1296.

The average basal metabolism for any subject during a given period is determined by adding together the total numbers of calories of heat produced per 24 hours for the entire period and dividing this number by the number of determinations in the period. The surface area of the dogs was determined by the Meeh's formula.<sup>13</sup> The surface area of the persons was determined by the DuBois and DuBois<sup>14</sup> Ht. Wt. chart. The number of calories produced per kg. of body weight is found by dividing the calories produced per 24 hours by the body weight in kilos. Fractions of calories are not recorded but results are given in the nearest whole numbers. Variations in body weight are recorded in the nearest .1 of a kg. Accuracy beyond this is superficial since log. tables were used in the computations and the retention of urine, or feces, would swing the balance either way to the value of .1 of a kg.



Fig. 1. *A simple muzzle for connecting dogs to the Benedict portable respiratory apparatus, or other open or closed respiratory systems.*



Fig. 2. *Showing dogs under experimentation in the metabolism room. The dog at the left is connected to the Benedict portable respiratory apparatus by means of the muzzle. The dog at the right is resting quietly in preparation for having its basal metabolism determined. The picture was taken at a four minute time exposure, showing the complete quietude of the animals*





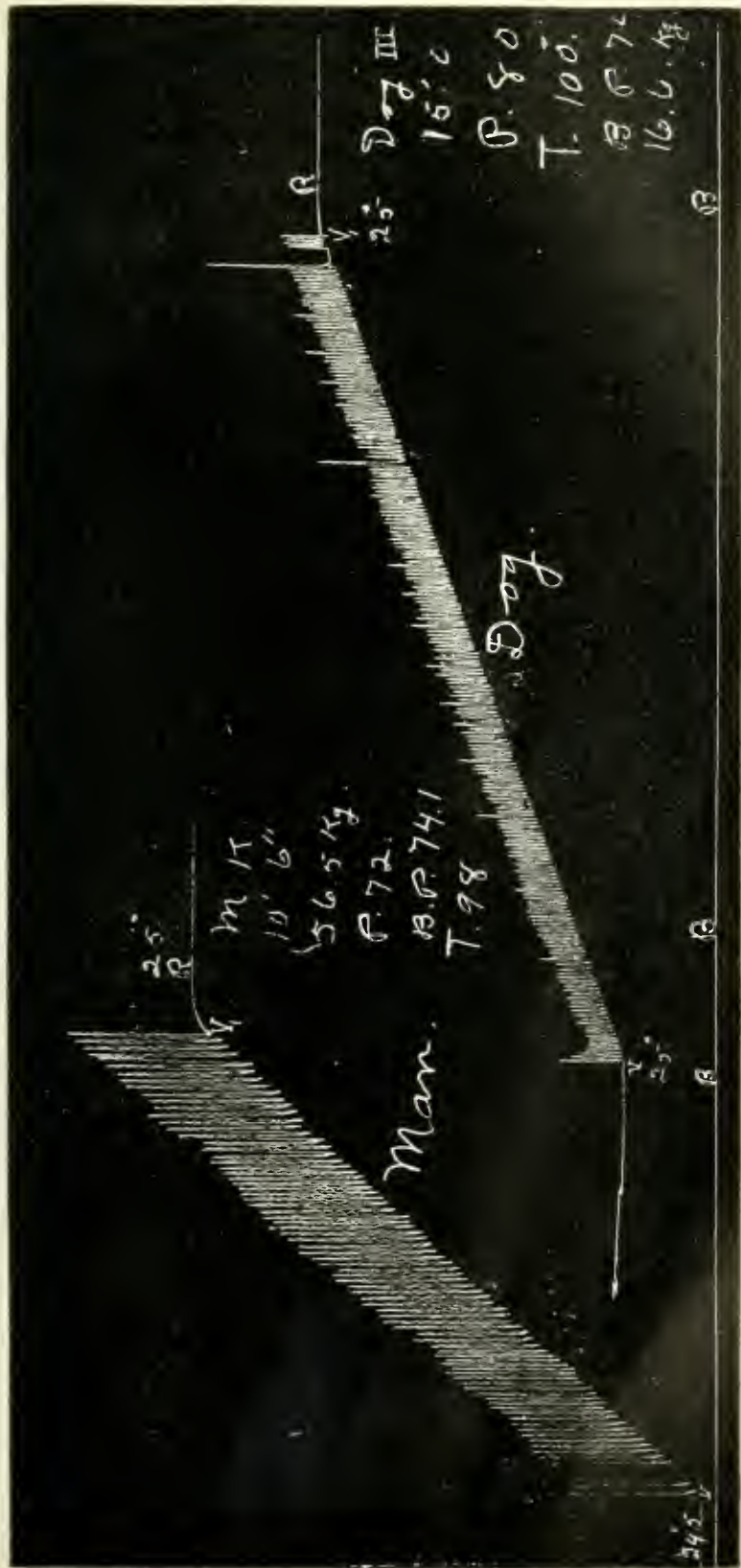


Fig. 3.





*Results on Man.*

*Subject M. K.* Basal metabolism estimations and other data tabulated were determined daily for the period of one year, excepting for eight days during the holidays, a three-day period when coughing interfered with the technique, and three or four single day periods when the data was rejected due to difficulties with the apparatus.

*The Control Period* for this subject extended through five months and thirteen days. The body weight during this period varied from 55 to 55.5 kilos. The pulse ranged from 66 to 82 beats per minute. The temperature varied between  $97^{\circ}.06$  —  $98^{\circ}.40$  F. The surface area, according to DuBois and DuBois Ht. Wt. chart was 1.63 sq. meters. The average basal metabolism for this period was 1375.2 calories per 24 hours. The average number of calories of heat produced per sq. meter of body surface for 24 hours was 843.7. The average body weight was 55.2 kilos. The average number of calories per average kg. of body weight per 24 hours was 24.91. The greatest deviation from the average heat production was 13.6% plus on April 4 and 8.3% minus on September 10. These extremes occur at widely different seasons and this may account for some of the difference as will be discussed later.

Since the pre-fasting period extends through changing seasons and the metabolic rate of this subject was slightly higher in the early spring than in mid-summer, it becomes obvious that a more accurate comparison of the pre- and post-fasting metabolism can be made by using as a control, not the entire six months period, but the ten weeks immediately preceding the fast. This has been done, and gives a slightly different average as can be seen from Table 6.

*The Fasting Period.* During the 15 days of fasting there was a loss of 7.9 kilos in body weight. This represented 14.3% of the initial body weight. The subject was 17 lbs. underweight at the beginning of the fast; extremely emaciated at the end of the fast. The average body weight during the fasting period was 50.3 kilos. The average basal metabolism during the 15 days of fasting was 1403.2 calories per 24 hours. The highest basal metabolism of this period occurred on the 4th day of the fast. On this day the total heat production per kg. of body weight was 9% higher than the highest metabolism of the control period. The lowest metabolism was reached on the last day of the fast. The total heat

production on this day was 1300 calories. The body weight was 47.6 kilos. The number of calories of heat produced per kg. of body weight was 27.5 calories. This is an increase of 2.6 calories over the average heat production per kg. of body weight during the six months control period. The average heat production for the 15 fasting days per average kg. of body weight was 27.8 calories, an increase of 12.6% above the control metabolism. The pulse rate remained normal; no change occurred in the body temperature during fasting.

*The Period After Fasting.* In determining the averages for this period the data for the first 5 days was rejected because the body weight was still below normal and the amount of food consumed was considerably below the daily food consumption of the control period.

*The Basal Metabolism.* The average number of calories of heat produced per 24 hours for the after-fasting period of six months excluding the first 5 days of the period was 1475.7 calories. This is an increase of 100.5 calories per 24 hours over the average of the 5½ months control period. The average body weight was 57.2 kilos. The average number of calories of heat per 24 hours per average kg. of body weight was 25.79, an increase of 3.1% over the 5½ months control period. The average heat production for the first two months of the period was 1465 calories. The average body weight was 56.2. The average number of calories per average kg. of body weight was 26.07, an increase of 6.1% over the basal metabolism of the 10 weeks preceding the fast. The metabolism for the last month (March, 1922) of this period was 1499 calories per 24 hours. The body weight was 57.3 kilos, the average number of calories per average kg. of body weight was 26.16. The average heat production for the first month of the control period (April, 1921) was 1427. The average body weight for that month was 55.2. The average number of calories per kg. of body weight was 25.86. The basal metabolism for Mar. (7th month after the fasting period) was 1.1% higher than for April of the pre-fasting period.

*Subject, F. H. — The Control Period* for this subject was only 2 weeks in duration. During this time 8 basal metabolism tests were made. The average number of calories of heat produced per 24 hours during this period was 1435.7. The body weight was somewhat variable, ranging from 59.5 to 61.8 kilos. The surface

area at the end of this period was 1.68 sq. metres. The average daily heat production per sq. meter of body surface was 854.1 calories. The temperature varied from  $96^{\circ}.80$  to  $97^{\circ}.20$ . The pulse rate was very constant, varying only between 56 and 60 beats per minute. The average body weight during this period was 61.1 kilos. The average number of calories of heat produced per average kg. of body weight per 24 hours was 23.4 calories. The variations in heat productions were not marked. The greatest increase above the average was 4.8% on November 11 and the greatest decrease was 3.5% below the average on November 1.

*The Fasting Period.* The loss in body weight during this period was only 6.3 Kilos, or 10.2% of the initial body weight. The basal metabolic rate was higher than normal until the 9th day of the fast. After this it was approximately the same as the normal. The highest metabolism of this period occurred on the 3rd fasting day. At that time it was 10.9% above the average for the control period. The metabolism on the last day of the fasting was 22.6 calories per kg. of body weight. The metabolism on the last day of the control period was 22.8 calories per kg. of body weight. The average daily heat production during the entire fasting period was 1403.9 calories. The average body weight was 58.4 kilos. The average heat production per average kg. of body weight was 24.02 calories, an increase of 2.6% above the average control metabolism.

The body temperature remained normal during the fast. The pulse rate on the last day of the fast was 8 beats less per minute than on the first fasting day.

On November 25th, the 7th day of the fast, the subject began eating non-nutritive cellulose wafers. He continued eating this substance daily until after the metabolism tests were made on December 1. The decrease in the basal metabolism on the day following the ingestion of cellulose was greater than the decrease which had occurred on any previous fasting day. It is interesting to note that the next determination made after the subject had stopped eating cellulose is somewhat higher in spite of the marked lowering in the pulse rate and the loss in body weight. This matter will be taken up in a later report.

*The Period After Fasting.* The results obtained from this subject are somewhat difficult to present because intermittent fasting days occurring the 5th, 7th and 8th weeks interrupted the con-

TABLE NO. 1  
Daily Data for Subject M. K.

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.	Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.
<b>1921 A. Control Period</b>					<b>1921</b>				
Apr.					May				
1	98	72	55	1416	20	97.8	72	55.5	1440
2	98.1	84	55	1529	21	97.8	74	55.4	1410
3	98	80	55	1470	22	98.1	72	55.2	1363
4	98.1	84	55.1	1563	23	97.8	72	55.2	1390
5	98	72	55.3	1497*	24	97.8	72	55.4	1320
6	98.1	72	55.1	1401	25	97.8	76	55.2	1310
7	98.1	76	55.4	1542	26	98	72	55.5	1396
8	98	68	55.4	1380	27	98.1	76	55.5	1402
9	98.1	70	55.4	1347	28	97.8	74	55.5	1310
10	98.4	74	55.5	1416	29	97.8	76	55.5	1343
11	98.1	70	55.5	1385	30	98	74	55.2	1360
12	98.4	72	55.5	1410	31	98	76	55.4	1390
13	98.4	72	55.3	1448	June				
14	98.3	74	55	1357	1	98.1	78	55.4	1420*
15	98.3	74	55	1332	2	98	76	55.4	1333
16	98.6	76	55	1425	3	98	76	55.4	1353
17	Mechanical		Difficulties		4	97.6	76	55.4	1360
18	98.4	72	55	1434	5	97.9	76	55.2	1320
19	98.4	72	55.3	1393	6	98	72	55.4	1360
20	98.4	74	55.5	1453	7	98.1	80	55.2	1420
21	98.1	72	55.5	1363	14	98.1	74	55.2	1340
22	98.4	76	55	1446	15	98.1	74	55.2	1359
23	98.4	76	55	1412	16	98.1	74	55.2	1367
24	98.4	78	55	1438	17	98	76	55.2	1310
25	98.6	80	55	1501	18	98	76	55.4	1361
26	Mechanical		Difficulties		19	97.8	72	55.4	1390
27	98.2	72	55.3	1398	20	97.8	74	55.2	1360
28	98.4	76	55.4	1466	21	98	74	55.4	1370
29	98.2	70	55.2	1367	22	97.8	74	55.5	1331
30	98.2	78	55.2	1390	23	97.8	74	55.5	1304
May					24	97.8	74	55.5	1312
1	98	78	55.4	1398	25	98	74	55.5	1461
2	97.6	76	55	1370	26	98	74	55.5	1434
3	97.6	76	55.4	1366	27	97.8	70	55.3	1367
4	97	76	55.2	1380	28	98.2	76	55.4	1491
5	98.1	80	55.4	1467*	29	98	72	55.4	1348*
6	98.1	76	55.4	1365	30	98	72	55	1330
7	98.2	76	55.2	1356	July				
8	98.4	76	55.2	1375	1	98	74	55.4	1400
9	98.2	78	55.2	1400	2	97.6	72	55	1371
10	98.5	78	55.5	1410	3	98	72	55	1340
11	98.4	76	55.5	1360	4	98	72	55	1300
12	98.3	76	55.5	1350	5	Mechanical		Difficulties	
13	98.4	78	55.5	1420	6	98	68	55	1290
14	98.2	80	55.5	1400	7	98.2	74	55	1450
15	98.4	80	55.5	1420	8	98	72	55	1354
16	97.9	72	55.5	1360	9	98.4	72	55	1310
17	97.6	66	55.2	1380	10	98	74	55	1400
18	98.1	72	55.4	1400	11	98.1	74	55	1388
19	97.6	76	55.5	1465	12	98.1	74	55	1360



TABLE NO. 1—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.	Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.
<b>1921</b>					<b>1921</b>				
July	<b>A. Control Period—Cont.</b>				Sept.				
13	98.1	74	55	1318	1	98	72	55.2	1342
14	98	74	55	1360	2	98	72	55.2	1315
15	98	76	55.1	1390	3	98	72	55.2	1315
16	98	76	55.1	1385	4	98	72	55.2	1327
17	97.8	72	55.2	1374	5	98	72	55.2	1327
18	97.8	74	55.2	1330	6	98.1	66	55.5	1302
19	97.8	78	55	1360	7	97.8	72	55.5	1279
20	98	74	55	1400	8	98.1	74	55.5	1330
21	97.8	74	55	1346	9	98.4	72	55.5	1361
22	98	74	55	1366	10	98.1	70	55.5	1260
23	98	72	55	1374	11	98.1	72	55.5	1270
24	98	72	55	1350	12	98.2	74	55.5	1288
25	98	78	55	1420	13	98.2	74	55.5	1344
26	97.8	72	55	1360					
27	97.8	74	55	1368	<b>B. Fasting Period</b>				
28	97.8	76	55	1348	14	98.1	72	55.5	1351
29	97.6	76	55	1370	15	97.6	72	54.6	1532
30	98	76	55	1390	16	98.1	74	53.4	1541
31	97.8	76	55	1390	17	98.4	80	52	1590
Aug.					18	98.2	70	50.8	1500
1	97.8	74	55	1342	19	98.4	70	50.7	1525
2	97.8	74	55.1	1346	20	98.2	75	50.3	1432
3	98.2	74	55.2	1325*	21	98.2	80	49.5	1370
4	98	72	55.1	1353	22	98.4	77	49.2	1348
5	98	78	55.5	1345	23	99	72	49	1305
6	98	76	55.4	1325	24	98.6	74	48.5	1316
7	98	74	55.4	1326	25	98.6	72	48.4	1330
8	98	76	55.4	1395	26	98.4	74	48.1	1300
9	98	78	55.4	1410	27	98.4	74	48	1308
10	98	78	55.4	1441	28	98.2	76	47.6	1300
11	98	72	55.4	1392					
12	98.1	72	55.2	1410	<b>C. Period After Fasting</b>				
13	98	78	55.1	1476	29	98.2	76	48.1	1236
14	98	76	55.1	1374	30	98.4	74	50.1	1266
15	98.1	72	55.1	1307	Oct.				
16	98	72	55.1	1330	1	98.4	74	51.6	1301
17	98	78	55	1330	2	98.4	74	53.2	1310
18	98.1	78	55	1400	3	98.2	76	53.5	1350
19	98.1	78	55	1401	4	98.3	76	54.7	1317
20	98	76	55	1330	5	98.2	75	55.8	1550
21	98.4	80	55	1380	6	98.4	76	55.5	1430
22	98	75	55	1340	7	98.4	74	55.4	1603
23	98.4	80	55	1366	8	98.4	76	55.5	1407
24	98.1	76	55	1324	9	98.3	76	55.4	1480
25	98	80	55	1361	10	98.3	76	55.4	1470
26	98.6	82	55	1445	11	97.8	76	55.5	1424
27	98.6	76	55	1360	12	98	76	55.6	1435
28	98.6	76	55	1372	13	97.8	76	55.6	1460
29	88.4	74	55	1267	14	97.8	76	55.6	1470
30	98	70	55.2	1310*	15	97.9	78	55.7	1497
31	98	74	55	1357					



TABLE NO. 1—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.	Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.
<b>1921</b>	<b>C. Period After Fasting—</b>				<b>Cont'd.</b>				
Oct.					Dec.				
16	97.9	78	55.7	1490	4	97.4	70	57.2	1428
17	97.9	76	55.8	1521	5	97.4	70	57.2	1404
18	97.9	76	55.8	1508	6	97.4	72	57.2	1438
19	98	78	55.8	1478	7	97.4	72	57.2	1407
20	98.6	78	55.8	1502	8	97.4	74	57.2	1472
21	98.6	78	55.9	1470	9	97.6	74	57.2	1443
22	98.4	74	55.9	1511	10	97.3	74	57	1449
23	98.4	74	55.9	1406	11	97	72	57	1402
24	98.2	78	55.9	1396	12	97.4	78	57	1440
25	Mechanical		Difficulties		13	97.6	78	57	1455
26	98.4	74	55.9	1468	14	98	78	57	1480
27	98.2	74	56	1541	15	97.8	76	57	1493
28	98.2	76	56	1535	16	97.6	74	57	1450
29	98.2	74	56	1483	17	97.4	76	57	1450
30	98.3	74	56	1490	18	97.8	78	57	1501
31	98.2	72	56	1397*	19	97.6	78	57	1530
Nov.					20	97.6	76	57.2	1434
1	98	74	56	1330	21	97.6	74	57.2	1491
2	98.2	70	56	1436	22	97.6	74	57.4	1499
3	98	70	56	1476	23	Mechanical		Difficulties	
4	98.1	74	56	1445	24	96.8	66	57.4	1370
5	98	72	56	1427	<b>1922</b>				
6	98	72	56	1403	Jan.				
7	98	74	56.2	1471	3	97.9	74	57.4	1420
8	97.6	72	56.3	1387	4	Bad Cold; Cough Inter-			
9	98	74	56.4	1470		ferred			
10	98	80	56.5	1500	5	Cough	Continued		
11	98.1	80	56.5	1472	6	Cough	Continued		
12	97.8	80	56.6	1424	7	97.6	70	57.4	1417
13	98	72	56.6	1520	8	97.8	74	57.4	1439
14	98	71	56.7	1487	9	97.8	74	57.4	1423
15	98	76	56.7	1494	10	97.8	74	57.4	1410
16	98.2	74	56.7	1488	11	97.8	74	57.4	1424
17	98.2	74	56.7	1476	12	97.8	76	57.2	1438
18	98.5	76	56.7	1501	13	97.6	74	57.2	1433
19	98.4	78	56.8	1442	14	97.6	76	57.2	1410
20	98.3	76	56.8	1496	15	97.6	76	57.2	1478
21	98.4	74	56.8	1450	16	Mechanical		Difficulties	
22	98.2	74	56.8	1467	17	97.6	80	57.2	1584
23	98.4	76	56.8	1524	18	97.6	74	57.2	1422
24	98.4	84	56.9	1496	19	97.2	76	57.2	1537
25	98.4	84	56.9	1571	20	97.2	72	57.2	1497
26	98.3	72	56.9	1440	21	97.2	72	57.3	1515
27	98.3	72	56.9	1505	22	97.4	70	57.3	1457
28	97.8	74	57	1400*	23	97.6	76	57.3	1471
29	97.4	74	57	1440	24	Mechanical		Difficulties	
30	97.6	72	57	1447	25	97.2	76	57.3	1455
Dec.					26	97.2	76	57.5	1380*
1	97.6	74	57	1470	27	97.6	76	57.5	1540
2	97.4	74	57.2	1424	28	97.4	78	57.5	1513
3	97.4	74	57.2	1438	29	97.4	76	57.5	1453

TABLE NO. 1—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.	Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.
<b>1922</b>	<b>C. Period</b>	<b>After</b>	<b>Fasting—</b>	<b>Cont'd.</b>					
Jan.				Mar.					
30	97.6	74	57.5	1486	1	97.8	76	57.5	1499
31	97.2	76	57.5	1504	2	98	76	57.5	1490
Feb.					3	97.8	72	57	1491
1	97.4	76	57.5	1480	4	98	74	57.3	1446
2	Mechanical	Difficulties			5	98	74	57.3	1440
3	97.6	80	57.5	1509	6	98	76	57.3	1435
4	97.8	76	57.5	1452	7	98	76	57.3	1532
5	Mechanical	Difficulties			8	98	80	57.3	1560
6	97.8	74	57.2	1444	9	98	80	57.3	1515
7	97.6	76	57.4	1418	10	98.2	80	57.5	1520
8	97.6	76	57.4	1417	11	97.6	82	57.5	1585
9	97.6	76	57.4	1429	12	97.8	76	57.5	1542
10	97.6	80	57.5	1532	13	98	76	57.5	1537
11	97.6	74	57.5	1491	14	98	82	57.5	1505
12	97.6	74	57.5	1460	15	97.8	78	57.5	1493
13	97.8	80	57.5	1557	16	97.6	76	57.5	1467
14	97.2	70	57.5	1400	17	98	82	57.3	1575
15	97.4	76	57.5	1493	18	97.8	76	57.3	1529
16	97.6	80	57.5	1535	19	97.8	76	57.5	1542
17	97.8	76	57.5	1494	20	97.8	76	57.2	1530
18	97.8	76	57.5	1480	21	97.6	82	57.2	1525
19	97.8	84	57.5	1570	22	97.6	82	57.2	1502
20	97.6	86	57.5	1536	23	97.6	80	57.3	1456
21	97.8	80	57.5	1526	24	97.6	76	57.3	1460
22	97.8	82	57.5	1520	25	97.6	76	57.3	1466
23	97.8	86	57.5	1600	26	97.6	76	57.5	1462
24	97.6	80	57	1542*	27	97.6	76	57.5	1438
25	97.8	76	57.5	1544	28	98	78	57.5	1534
26	97.6	80	57.5	1500	29	97.6	76	57.3	1444
27	97.6	76	57.5	1511	30	97.6	76	57.3	1448
28	97.8	76	57.5	1542	31	98	84	57.3	1518

\*First Day of Menstruation.

tinuity of this period. The data from January 10th to January 29th was rejected from the calculation of the averages because the conditions, due to these fasts, were not comparable to the conditions of the control period. The conditions were again normal from January 29th to February 15th, when the period ended. The first determination of the basal metabolism of this period was made on the 2nd day after eating. This result is not included in the computation of the average because the body weight had not reached that of the control period. The number of calories of heat produced per 24 hours on the 2nd day after fasting is 46 calories less than on the last day of the fast in spite of the fact

that the body weight had increased 2.5 Kilos. On the 5th day after eating the body weight had returned to normal. At the end of the first week of this period the body had gained 18.2 lbs. The weight on December 11th was 2 kilos more than it was at the end of the control period. The body weight continued to increase throughout this period, at first rapidly, later more gradually. At the end of the period (February 15th) the body weight was 3.2 Kilos. more than it was at the beginning of the fast period. The average body weight for this period was 63.7 Kilos. The food eaten was as near that of the control period as could be estimated without making quantitative determinations.

*Basal Metabolism.* The average heat production for this period was 1526.3 calories per 24 hours. The average number of calories of heat produced per average kg. of body weight per 24 hours was 23.9. This was an increase of 2.1% above the average heat production per average kg. of body weight during the control period. This period should have been continued for a longer time but the subject refused to eat the amount of food that was consumed during the control period because of the marked tendency to gain in weight.

The body temperature remained the same as during the control period. There was a slight increase in the pulse rate. Bowel movements were more regular than during the control period.

### *Results on Dogs*

*Subject, Dog No. 1. Young female. The Control Period.* Eight basal metabolism determinations were made on this dog from March 21st to April 9th inclusive. The average heat production for this period was 360.1 calories per 24 hours. The greatest increase above this average was less than 1% (on March 21 and April 5.) The greatest decrease was 1.6% below the normal on April 2nd. The body weight was very constant (10.2 Kilos.), varying only .1 of a kg. during this period. The average number of calories of heat produced per 24 hours per average kg. of body weight was 35.3. The surface area computed by Meeh's formula was .526 sq. meters. The number of calories of heat produced per sq. meter of body surface per 24 hours was 686. The maintenance diet (250 g. meat, 250 cc. milk and 100 g. bread) furnished approximately 861 calories.

*The Fasting Period* (April 9 to May 15) was 37 days in duration. The total loss of body weight was 4 Kilos. This is 39.3%

TABLE NO. 2  
Daily Data for Subject F. H.

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Cal. per 24 Hrs.	Remarks
<b>1921</b>	<b>A. Control</b>	<b>Period</b>			
Nov. 3	97.4	56	59.5	1385	Surface Area 1.68 sq. meters.
5	97	56	61	1405	
7	97.2	56	61.7	1436	
9	96.8	58	61	1483	
11	97.4	60	61.4	1490	
13	97	56	61.2	1423	
15	96.8	60	61.2	1452	
17	97.2	60	61.8	1412	10:00 P. M. last meal before fasting period.
	<b>B. Fasting</b>	<b>Period</b>			
19	96.4	56	61.2	1523	Second fast day.
21	96.4	54	60.3	1592	Fourth Fast Day.
23	96.2	56	59	1564	Sixth fast day.
25	97	60	58.6	1494	Eighth fast day—Began eating non-nutritive cellulose.
27	96.6	56	57.7	1355	Tenth fast day.
29	96.6	54	57.8	1294	Twelfth fast day.
Dec. 1	96.4	56	57.1	1212	Fourteenth fast day—Stopped eating cellulose.
3	96.4	48	55.5	1258	Sixteenth fast day.
	<b>C. Period after Fasting</b>				
5	97	62	58	1235	
7	97	64	60.7	1407	
11	96.6	64	63.8	1461	
13	96.8	64	62	1491	
15	96.6	70	63.1	1867	
17	96.8	68	63.1	1465	
19	96.8	70	63.3	1513	
21	97	70	63.7	1551	
24	97	70	63.4	1532	
<b>1922</b>					
Jan. 3	97	60	64.2	1436	Fasted all day.
5	96.8	58	64	1433	
10	96.4	58	63.5	1491	No food Jan. 7.
14	97.2	62	63.5	1545	No food Jan. 10 and 11.
17	96.8	56	63.8	1334	
21	96.2	58	64	1411	No food Jan. 19.
24	97.4	58	64	1392	No food Jan. 21.
27	96.4	58	63.9	1438	No food Jan. 24.
29	97	62	63.9	1545	Three meals daily.
31	96.8	58	63.9	1520	Three meals daily.
Feb. 2	96.8	60	64.7	1509	Three meals daily.
4	96.8	66	64.8	1566	Three meals daily.
8	97	62	64.9	1566	Three meals daily.
11	96.8	62	65	1583	Three meals daily.
14	96.2	64	65	1503	Three meals daily.

of the initial weight. The average basal metabolic rate during the fasting period was 305.3 calories per day. The average body weight was 8.04 kilos. The average number of calories of heat per average kg. of body weight was 37.9. The basal metabolism on the last day of the fast was 40.6 calories per kg. of body weight, an increase of 15.01% above the average control metabolism.

*The After-Fasting Period (May 16 to March 1).*

*The Basal Metabolism.* Since the after-fasting period of this dog was  $9\frac{1}{2}$  months in length and extended through changing seasons and varying physiological conditions, the data becomes much more significant and comprehensive if the period is subdivided into its significant aspects and the averages for these significant subdivisions presented and interpreted in terms of the physiological condition which seems to predominate for the given time. Presentation of the data according to this plan gives us (1) a period of one month when the body is rapidly gaining the weight lost during the starvation. (2) A period of about six weeks during which the body weight was quite constant and approximately the same as during the control period. (3) Various phases of the estrual cycle.

The average daily heat production from May 19th (dog retained food and showed no disturbed symptoms) until June 19th (body weight normal) was 383.4 calories. The average body weight was 9.1 kilos. The average number of calories of heat produced per average kg. of body weight per 24 hours was 42.1, an increase of 19.30% above the control or pre-fasting metabolism. June 19th to August 2nd represents the time when the body weight of the dog was approximately that of the control period. The difference was less than .1 of a kg. at most. The body weight and other conditions are therefore quite comparable to the control period, except that the food had been reduced so that the dog was receiving 132 calories less than the maintenance diet for the control period. The average daily heat production from June 19th to August 2nd was 407.5 calories. The average body weight was 10.2 kilos. The average heat production per average kg. of body weight was 39.9 calories. This is an increase of 13% above the normal control metabolism. The remainder of the period, August 2nd to February 28th inclusive, has been subdivided into several periods in order to determine the effect of the various phases of the estrual cycle on the metabolism.



*The Basal Metabolism during the Estrual Cycle.* The estrual cycle occurred twice during the after fasting period of this dog. The duration of the first cycle (August 4 to August 26) seemed to be 23 days. The average heat production per 24 hours during this period was 415.4 calories. The body weight was 10.4 kilos. The average heat production per average kg. of body weight was 39.4 calories. The average heat production per 24 hours for the 23 days immediately preceding the rut was 400.1 calories. The average body weight during this time was 10.23 kilos. The average heat production per average kg. of body weight for the 23 days preceding the estrual cycle was 39.2 calories. The basal metabolism during this estrual season in this dog was exactly the same as the metabolism of an equal number of days immediately preceding the rut.

The average number of calories of heat produced per day for the second estrual season (February 14 to February 28) was 482.8. The average body weight was 13.3 kilos. The average heat production per average kg. of body weight was 42.7 calories. The average metabolism for the 15 days immediately preceding this rut season was 499.5 calories per day. The average body weight was 11.2 kilos. The average number of calories of heat produced per average kg. of body weight was 44.5, an increase of 4% above the rut metabolism. The dietary from August 20 to February 28 contained 643 calories.

. . .

#### *Dog No. 2, Young Male.*

*The Control Period (July 29th to September 17th).* During this period basal metabolism determinations were made daily with but few interruptions. The average daily heat production was 465.3 calories. The greatest variation from this was an increase of 10% on July 4th and a decrease of 9.8% on September 3rd. The relatively constant body weight for this period was 12.4 kilos. The average number of calories of heat produced per kg. of body weight per 24 hours was 37.54. The surface area was .60 sq. meters. The average number of calories per sq. meter of body surface was 775. The pulse rate varied from 72 to 90. The body temperature from 101°.20—100° F.

*The Fasting Period.* During this period (40 days) the dog lost 5.2 kilos. of body weight. This was 42% of the control weight. The pulse rate decreased as the fast progressed, reaching the lowest rate of 44 beats per minute on October 19th, the 33rd day



TABLE NO. 3  
Daily Data for Subject Dog No. 1

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
<b>1921</b>						
<b>Mar.</b>	<b>A.</b>	<b>Control</b>	<b>Period</b>			
21	100.8	50	10.3	26	363	100 g. bread, 250 g. meat, 250 c.c. milk
24	101	54	10.2	26	361	Food as above
31	100.8	48	10.2	28	360	Check test 364. " " "
<b>Apr.</b>						
2	101	48	10.3	20	354	" " "
3	101	52	10.3	20	359	" " "
5	100.8	60	10.2	20	363	" " "
6	100.8	58	10.3	22	361	" " "
8						Last feeding at 10:00 A.M.
	<b>B.</b>	<b>Fasting</b>	<b>Period</b>			
9	101	54	10.2	26	313	1st Fast Day
10	100.6	56	9.9	32	397	2nd " "
11	100.8	60	9.6	17	335	3rd " "
12	100.8	58	9.5	18	338	Depressed. 4th " "
13	100.8	65	9.4	16	347	Depressed. 5th " "
14	100.9	70	9.2	17	366	6th " "
15	101.1	60	9.2	16	363	7th " "
16	101.2	66	9	16	347	Check test 347. 8th " "
17	101.8	72	9	17	371	9th " "
18	101.4	66	8.8	15	301	10th " "
19	100.8	53	8.7	14	286	11th " "
20	100.8	53	8.6	14	315	12th " "
21	100.6	50	8.5	15	299	Check test 302. 13th " "
22	100.6	51	8.4	14	296	14th " "
23	100.6	54	8.3	20	324	15th " "
24	100.7	52	8.2	13	343	16th " "
25	100.6	50	8.2	13	307	17th " "
26	100.6	58	8.1	14	286	18th " "
27	100.1	50	8.1	12	272	19th " "
28	100.9	50	8.1	14	272	20th " "
29	100.8	45	8	12	279	Enema given. 21st " "
30	100.8	45	7.8	15	280	22nd " "
<b>May</b>						
1	101.6	54	7.7	13	284	23rd " "
2	101.6	51	7.6	11	272	24th " "
3	101.6	45	7.6	14	277	25th " "
4	101.6	53	7.4	13	282	26th " "
5	101.2	54	7.4	12	261	27th " "
6	101	43	7.3	12	282	28th " "
7	102.5	114	7.2	26	332	Depressed; vomited. 29th " "
8	102	110	7.1	13	347	Enema given. 30th " "
9	101.3	66	7	12	295	31st " "
10	100.8	90	6.9	11	278	32nd " "
11	101	102	6.8	11	282	33rd " "
12	101.2	86	6.7	11	270	34th " "
13						Mechanical Difficulties.
14	101		6.5	12	264	36th " "
15	101		6.4	12	271	Moribund. 37th " "
16	100.2	66	6.4	10	260	Fed 50 c.c. milk; vomited.
17	101.6	64	6.3	17	321	Vomited after each feeding.

TABLE NO. 3—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
May 18	101	62	6.2	12	300	Began to retain food; 100 g. bread, 250 g. meat, 250 c.c. milk
19	101.4	66	6.3	16	311	Vomiting ceased. Food as above
20	101.4	68	7	16	391	" " "
21	101.2	60	7.4	26	345	Dog apparently in good condition. " " "
22	101.2	66	7.7	22	354	" " "
23	101.4	62	8	25	360	" " "
24	101.4	62	8.1	25	379	" " "
25	101.4	66	8.3	23	355	" " "
26	100.8	70	8.6	18	357	Check test 360. " " "
27	101	68	8.7	25	397	" " "
28	100.9	68	8.8	23	376	" " "
29						Mechanical Difficulties.
30	101	70	8.9	25	387	" " "
31	100.8	68	9	25	368	" " "
June 1	100.4	70	9.1	18	381	" " "
2	100	66	9.2	18	387	" " "
3	100.1	68	9.3	17	389	" " "
4	101	69	9.4	18	366	" " "
5	101	68	9.5	17	414	" " "
6	101	68	9.3	18	414	" " "
7	101	70	9.2	18	375	Vomited " " "
8	101.4	70	9.2	16	393	" " "
9	101.4	62	9.3	30	393	" " "
10	101.6	58	9.5	20	331	" " "
11	101.2	60	9.6	30	381	" " "
12	101.2	58	9.6	30	400	Check test 402. " " "
13	101	66	9.7	15	365	" " "
14	101.2	64	9.8	34	416	" " "
15	101.2	66	9.9	34	399	Check test 400. " " "
16	100.6	66	10	36	408	Did not eat all food. " " "
17	100.6	62	10	34	395	" " "
18	100.6	62	10.2	40	423	Did not eat all food.
19	100.6	64	10.3	40	412	Body weight back to normal. " " "
20	101.6	66	10.2	34	423	" " "
21	100.8	76	10.3	40	412	Did not eat all food.
22	100.8	70	10.2	24	455	Depressed. " " "
23	101.1	60	10.2	35	424	" " "
24	101.1	64	10.3	24	412	Did not eat all food.
25	101.2	68	10.2	35	413	250 g. meat, 75 g. bread, 250 c.c. milk
26	100.6	66	10.2	28	415	Food as above
27	100.9	66	10.2	29	395	" " "
28	100.8	68	10.2	22	425	" " "
29	100.6	68	10.2	30	448	" " "
30	101	68	10.2	16	445	" " "
July 1	101	68	10.2	24	445	" " "
2						Mechanical Difficulties.
3	101	64	10.2	22	418	" " "

TABLE NO. 3—*Continued*

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
July						
4	101	68	10.2	30	433	Food as above
5	100.6	72	10.2	22	415	" " "
6	100.6	72	10.2	26	411	" " "
7	101	64	10.3	26	371	" " "
8	101	72	10.2	28	354	" " "
9	101.3	72	10.2	31	401	" " "
10	101	64	10.2	24	402	Check test 406.
11	101	66	10.2	16	411	" " "
12	101	68	10.2	22	413	" " "
13	101.6	66	10.2	30	415	" " "
14	100.8	66	10.2	15	401	" " "
15	100.4	68	10.2	15	371	" " "
16	100.4	66	10.2	16	354	" " "
17	100.5	63	10.2	18	354	" " "
18	100.4	62	10.2	14	371	" " "
19	100.8	66	10.2	21	411	" " "
20	100.6	66	10.3	16	410	" " "
21	100.8	68	10.2	16	415	" " "
22	100.6	68	10.3	16	400	" " "
23	100.8	62	10.2	18	395	" " "
24	100.8	66	10.2	18	412	200 g. meat, 75 g. bread, 250 c.c. milk
25	101	66	10.3	18	385	Food as above
26	100.6	70	10.2	20	411	" " "
27	100.6	70	10.3	20	411	" " "
28	100.8	58	10.2	40	397	" " "
29	100.9	68	10.3	40	408	" " "
30	101.2	67	10.2	21	423	" " "
31	100.6	68	10.3	18	415	" " "
Aug.						
1	100.4	66	10.3	18	402	" " "
2	100.6	66	10.4	18	410	" " "
3	100.6	66	10.3	18	420	" " "
4	101	72	10.4	20	435	Rut appeared.
5	101	72	10.4	28	425	" " "
6	100.4	74	10.4	17	412	" " "
7	100.4	66	10.4	16	412	" " "
8	100.4	64	10.3	17	415	" " "
9	100.2	66	10.3	16	400	" " "
10	100.6	66	10.3	16	380	" " "
11	100.4	66	10.3	16	412	" " "
12	100.6	68	10.4	16	427	" " "
13	100.4	74	10.4	18	392	" " "
14	100.4	66	10.4	24	404	" " "
15	100.6	68	10.4	16	382	Very drowsy.
16	100.6	68	10.4	15	400	" " "
17	101	61	10.4	16	415	" " "
18	101	66	10.4	20	438	" " "
19	100.2	70	10.4	20	431	" " "
20	100.2	66	10.4	30	450	200 g. meat, 50 g. bread, 250 c.c. milk
21	101	66	10.4	16	413	" " "
22	100.8	68	10.4	16	423	" " "
23	100.4	70	10.4	16	421	" " "

TABLE NO. 3—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
Aug.						
24	100	66	10.3	16	420	Food as above
25	100.8	66	10.4	20	423	" " "
26	100.8	62	10.4	20	432	Rut ceased. " " "
27	100.8	64	10.4	20	389	" " "
28	100.8	64	10.5	20	342	" " "
29	101.2	56	10.4	20	252	" " "
30	100.1	54	10.4	20	340	" " "
31	100.6	50	10.4	23	340	" " "
Sept.						
1	101	57	10.4	14	356	" " "
2	101.2	66	10.4	24	370	" " "
3	101.6	70	10.4	24	438	" " "
4	101.4	64	10.4	24	435	" " "
5	101.1	62	10.5	20	435	Slight amount of shivering. " " "
6	101.2	70	10.5	22	443	" " "
7	100.6	68	10.4	16	417	" " "
8	100.8	59	10.4	16	355	" " "
9	100.1	60	10.4	16	337	" " "
10	101	80	10.5	14	403	" " "
11	100.8	66	10.5	14	368	" " "
12	100.9	64	10.4	16	377	" " "
13	100.9	60	10.5	16	376	Slight amount of shivering. " " "
14	100.7	70	10.5	16	359	" " "
15	100	66	10.5	16	365	" " "
16	100	60	10.5	15	369	Check test 369. " " "
17	100.1	64	10.4	15	394	" " "
18	100	60	10.4	15	381	" " "
19	100.1	66	10.4	16	364	" " "
20	100	60	10.4	16	418	" " "
21	100.1	62	10.3	16	342	" " "
22	100.1	68	10.3	16	350	" " "
23	100	66	10.4	16	369	" " "
24	100	66	10.4	16	371	" " "
25	100.1	66	10.4	16	370	" " "
26	100.2	66	10.4	16	360	" " "
27	100	68	10.4	15	373	" " "
28	100.1	66	10.4	16	370	" " "
29	100.5	66	10.4	16	372	" " "
30	100	64	10.4	16	376	" " "
Oct.						
1	100.2	64	10.4	16	373	" " "
2	100.2	62	10.4	16	371	" " "
3	100.1	64	10.4	15	373	" " "
4	100	64	10.4	16	375	" " "
5	100.2	70	10.4	16	405	Slight amount of shivering. " " "
6	100	64	10.4	16	360	" " "
7	100	66	10.4	16	360	" " "
8	100.2	70	10.5	16	390	" " "

TABLE NO. 3—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
Oct.						
9	100.1	70	10.5	16	372	Food as above
10						Mechanical difficulties.
11	100.1	66	10.5	16	379	" " "
12	100.2	72	10.5	16	376	" " "
13	100	72	10.5	16	413	" " "
14	100	76	10.5	18	400	" " "
15	100	80	10.5	18	422	" " "
16	100	80	10.6	20	422	" " "
17	100	84	10.6	16	386	" " "
18	100.2	80	10.6	15	382	" " "
19	100	74	10.6	18	370	" " "
20	100.4	78	10.6	16	395	" " "
21	101	78	10.6	16	415	" " "
22	100.5	66	10.6	16	398	" " "
23	101.1	62	10.6	16	372	" " "
24	100	58	10.7	18	369	" " "
25						Mechanical difficulties.
26	100	64	10.7	20	370	" " "
27	100.6	72	10.8	16	399	Check test 399.
28	100.2	78	10.8	16	401	" " "
29	100.2	78	10.8	16	400	" " "
30	100.2	78	10.9	16	386	" " "
31	100	74	10.9	18	399	" " "
Nov.						
1	101	82	10.9	20	416	" " "
2	100.8	84	10.9	20	429	" " "
3	100.8	68	10.9	16	393	" " "
4	100.6	54	10.9	16	393	" " "
5	101	54	10.9	16	393	" " "
6						No determination.
7	100.8	62	10.9	16	400	" " "
8	100.1	62	10.8	18	406	" " "
9	100.1	54	10.8	16	406	" " "
10	100.1	68	10.7	16	444	" " "
11	101	60	10.7	16	440	" " "
12	100.8	60	10.7	16	415	" " "
13	100.1	66	10.7	16	406	" " "
14	101	66	10.7	16	448	Diarrhoea
15	101	68	10.7	18	459	" " "
16	101	56	10.6	18	432	" " "
17	100.5	55	10.7	18	450	" " "
18	100	64	10.7	20	469	Check test 471.
19	100.8	68	10.7	16	476	" " "
20	100.8	66	10.8	29	423	" " "
21	100.1	54	10.8	16	431	" " "
22	100.2	68	10.8	16	488	" " "
23	101	58	10.7	20	487	" " "
24	101	60	10.8	18	473	" " "
25	101	80	10.8	18	435	" " "
26	101	80	10.8	16	400	" " "
27	101	76	10.8	16	435	" " "
28	101	62	10.8	16	485	" " "

TABLE NO. 3—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
Nov.						Food as above
29	100.8	64	10.8	16	440	" " "
30	100.8	52	10.8	16	491	" " "
Dec.						
1	101	53	10.8	20	463	Check test 468. " " "
2	100.8	66	10.9	12	450	" " "
3	100.1	64	10.8	12	444	" " "
4	101	60	10.8	16	521	Check test 521. " " "
5	101	60	10.8	12	512	" " "
6	101	60	10.8	12	500	" " "
7	101	66	10.8	16	521	" " "
8	101.5	54	10.9	18	515	" " "
9	101.5	60	11	16	511	" " "
10	101	60	10.9	16	501	" " "
11	101	66	10.9	20	477	" " "
12	101	66	10.8	16	474	" " "
13	101	66	10.9	16	500	" " "
14	100.8	66	10.9	16	477	" " "
15	100.1	72	10.9	18	540	" " "
16	100.1	72	10.9	16	546	" " "
17	101	74	10.8	16	528	" " "
18	101	60	10.8	16	490	" " "
19	101	75	10.8	16	521	" " "
20	101	72	10.8	18	486	" " "
21	101	72	10.8	18	492	" " "
22	101	74	10.8	16	478	" " "
23	101	66	10.9	16	482	" " "
1922						
Jan.						
3	101	54	10.6	16	455	" " "
4	101	62	10.6	18	460	" " "
5	101	68	10.7	16	401	" " "
6	101	70	10.7	16	484	" " "
7	101	68	10.7	16	454	" " "
8	101	68	10.7	16	403	" " "
9	100.8	68	10.8	18	431	" " "
10	101	80	10.8	20	482	" " "
11	101	85	10.8	16	496	" " "
12	101	62	10.8	16	426	" " "
13	100.8	66	10.8	12	485	" " "
14	101	64	10.8	16	443	" " "
15	101	72	10.8	18		B. M. R. rejected. " " "
16	101	68	10.8	16		" " " " " " " " " " " " "
17	101	68	10.9	16	487	" " "
18	101	58	10.9	16	475	" " "
19	100.8	62	10.9	16	478	Check test 478. " " "
20	100.8	54	11	16	454	Check test 457. " " "
21	100.5	54	11	16	457	" " "
22	100.8	60	11	18	392	" " "
23	100.8	68	11	18	482	" " "
24	100.8	68	11	18	482	" " "
25	100.8	64	11	16	437	" " "
26	100.8	62	11.1	18	428	" " "



TABLE NO. 3—*Continued*

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
Jan.						
27	100	60	11.1	18	454	Food as above
28	100	72	11.1	18	480	" " "
29	100.4	60	11.2	20	482	" " "
30	100.6	66	11.2	16	445	" " "
31	100	60	11.2	16	514	Check test 445. " " "
Feb.						
1	100	72	11.3	16	479	" " "
2	100	60	11.2	16	497	Check test 498. " " "
3	100.2	64	11.3	16	525	" " "
4	100.2	64	11.2	16	485	" " "
5						Basal metabolism deter-
6	99.9	58	11.2	14	524	mination discarded. " " "
7	99.8	64	11.3	16	500	" " "
8	100	60	11.3	16	507	Check test 523. " " "
9	100	52	11.3	16	539	" " "
10						Mechanical difficulties. " " "
11	100	58	11.3	18	487	" " "
12	101.2	68	11.3	16	517	" " "
13	100.8	76	11.3	14	512	" " "
14	100	70	11.3	16	439	Rut began. " " "
15	100.2	72	11.3	16	530	Check test 513. " " "
16	00.2	78	11.3	16	451	" " "
17	100.2	64	11.3	18	453	Check test 458. " " "
18	100	58	11.3	18	479	" " "
19	101	72	11.3	18	496	" " "
20	100.4	72	11.2	16	437	Check test 434. " " "
21	100.4	68	11.3	18	448	" " "
22	100	62	11.3	18	448	Check test 450. " " "
23	100.2	64	11.3	18	517	Check test 523. " " "
24	100.4	68	11.3	16	498	Check test 501. " " "
25	106.6	60	11.3	18	524	" " "
26	100.6	68	11.3	18	501	Check test 505. " " "
27	100.2	64	11.3	18	528	" " "
28	100.1	64	11.3	18	493	" " "

of the fast. Following this there was a slight increase. There was no change in the body temperature during the fasting.

The basal metabolism slightly increased during the 2nd and 3rd day of the fast, reaching 7% above the average normal control metabolism on the 3rd day. After this there was decrease in the number of calories as the body weight diminished, but even during the lowest level of the fasting (40th day) the number of calories of heat produced per kg. of body weight was higher than the level of the lowest control metabolism. The average number of calories of heat produced per day during this period was 391.8. The average body weight was 9.78 kilos. The aver-

age heat production per kg. of body weight per 24 hours was 40.0 calories.

*Period after Fasting.* The dog suffered severe gastric and other disturbances during the first three days of this period; therefore the data for these days was rejected in the determination of averages. After the 4th day the dog was fed on the maintenance diet of the control period until its body weight had returned to normal.

*The Basal Metabolism.* The average heat production from the time that food was retained (November 1) until the original body weight had been reached (January 4) was 483.7 calories per 24 hours. The average body weight was 10.7 kilos. The average heat production per kg. of body weight per 24 hours was 45.2, an increase of 20.4% over the control average. The average heat production from the time that the normal body weight had been regained until experimentation on the dog ceased (April 6) was 515.6 calories. The average body weight during this time was 12.9 kilos, an increase of .5 kg. above the normal. The average number of calories of heat produced per average kg. of body weight per 24 hours was 39.98, an increase of 6.5% above the control period, 5½ months after the fast had ceased.

*Dog No. 3, Old Female (Multipara).*

*The Control Period (October 17 to December 23).* During this period 24 daily metabolism tests were made. The average daily heat production for this period was 515.5 calories. The greatest deviation from the average metabolism was an increase of 5.3% on October 27th and a decrease of 8.4% on October 30th. The body weight varied from 11.8 to 12.2 kilos. The average body weight was 12 kilos. The average heat production per average kg. of body weight per 24 hours was 42.9 calories. The temperature varied from 100°F. to 101°F. Pulse ranged from 60 to 74. This dog was fatter than either of the others and apparently much older. The surface area was .587 sq. meters. The average heat production per sq. meter of body surface was 941 calories per 24 hours.

*The Fasting Period.* The loss in body weight was 4.5 kilos., 45% of its original weight. The average body weight during this period was 8.73 kilos. The average heat production per 24 hours was 379.4 calories. The average heat production per kg. of body weight was 43.45, 1.2% higher than the average for the control

TABLE NO. 4  
Daily Data for Subject Dog No. II

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
<b>1921</b>	<b>A.</b>	<b>Control Period</b>				
July						
29	101.2	88	12.4	28	504	250 g. meat, 100 g. bread, 250 c.c. milk
30	101.	90	12.4	28	508	Slight movement. Food as above
31	101.	88	12.3	15	495	Check test 499. " " "
Aug.						
1	101.	76	12.4	25	485	" " "
2	101.	76	12.4	24	435	" " "
3						Data rejected. Restless.
4	101.4	76	12.5	25	515	Check test 510. " " "
5	101.	76	12.4	23	495	" " "
6	100.6	76	12.4	23	440	" " "
11	101.2	72	12.5	26	476	Check test 476. " " "
12	101.2	84	12.4	35	491	" " "
13	101.	78	12.3	20	473	" " "
14	101.	72	12.3	20	444	" " "
15	100.6	72	12.3	26	481	Check test 484. " " "
16	100.6	76	12.4	24	450	" " "
20	101.1	80	12.4	24	462	" " "
21	101.1	80	12.3	26	493	" " "
22	100.	90	12.4	18	473	" " "
23	101.	80	12.4	26	450	" " "
24	101.	80	12.3	14	476	" " "
25	101.	76	12.4	18	470	" " "
26	101.	80	12.4	20	491	" " "
27	101.2	68	12.4	28	482	" " "
28	101.2	70	12.5	55	486	Rapid resp. due to humidity
29	101.	80	12.4	20	486	Food as above
30	101.	70	12.3	20	490	" " "
31	101.1	70	12.4	58	452	" " "
Sept.						
1	100.8	68	12.4	42	434	" " "
2	100.8	64	12.4	16	424	Check test 429. " " "
3	100.8	64	12.4	16	420	" " "
4	101.	70	12.3	18	437	" " "
5	100.9	72	12.3	24	440	" " "
6	101.	74	12.4	22	463	" " "
7	100.9	76	12.4	20	470	" " "
8	101.	72	12.3	16	435	" " "
9	101.	60	12.3	16	417	" " "
10	100.8	80	12.4	15	420	" " "
11	101.2	78	12.4	14	464	" " "
12	100.	80	12.3	14	423	" " "
13	100.	80	12.4	15	486	" " "
14	100.6	70	12.4	16	489	" " "
15	101.	74	12.4	16	465	" " "
16	100.3	72	12.4	15	454	Last feeding before fast at 10:00 A.M.
	<b>B.</b>	<b>Fasting Period</b>				
17	100.4	80	12.4	15	444	1st Fast Day
18	100.4	67	11.9	14	470	2nd " "
19	100.4	70	11.9	14	499	3rd " "
20	100.6	70	11.7	16	459	Check test 456. 4th " "

TABLE NO. 4—(Continued)

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
1921						
Sept.						Fast Day
21	100.2	60	11.7	10	439	5th " "
22	100.2	60	11.4	12	454	6th " "
23	100.3	68	11.2	12	459	7th " "
24	100.2	60	11.1	10	461	8th " "
25	100.2	55	10.9	10	400	9th " "
26	100.4	60	10.8	8	429	10th " "
27	100.4	60	10.6	10	440	11th " "
28	100.2	58	10.6	10	410	12th " "
29	100.2	58	10.3	8	409	13th " "
30	100.4	60	10.1	8	457	14th " "
Oct.						
1	100.2	60	10.2	8	411	15th " "
2	100.4	58	10.3	10	391	16th " "
3	100.4	60	10.2	8	429	17th " "
4	100.2	55	10.1	10	490	18th " "
5	100.2	55	10.	10	588	Slight Amount of Shivering. 19th " "
6	100.4	66	9.9	8	508	20th " "
7	100.2	58	9.8	8	376	21st " "
8	100.2	58	9.6	8	362	22nd " "
9	100.	56	9.4	8	347	23rd " "
10	100.2	56	9.4	8	335	Very alert and playful. 24th " "
11	100.4	48	9.3	8	317	25th " "
12	100.3	52	9.2	7	346	26th " "
13	100.8	52	9.	8	355	27th " "
14	100.4	52	8.9	7	320	28th " "
15	100.4	47	8.9	8	352	Check test 358. 29th " "
16	100.2	47	8.8	8	340	30th " "
17	100.4	50	8.7	8	345	31st " "
18	100.2	48	8.6	7	319	32nd " "
19	100.2	44	8.5	7	325	33rd " "
20	100.4	54	8.4	8	324	34th " "
21	101.	51	8.2	8	314	35th " "
22	101.	51	8.2	7	308	Check test 309. 36th " "
23	101.	54	7.8	8	326	37th " "
24	101.5	50	7.9	8	327	38th " "
25						Mechanical difficulties. 39th " "
26	101.	57	7.8	7	305	40th " "
27	101.	55	7.7	7	283	Fed 20 g. meat immediately after taking determination. Vomited. 41st " "
28	C. 101.	Perio 78	d After 7.5	Fast 15	ing 326	Vomited, convulsions. Fed 40 c.c. milk.
29	103.	88	7.3	13	411	Fed 50 c. c. milk, 25 g. bread.
30	103.	90	7.2	12	425	50 c.c. milk; convulsions, vomiting ceased.
31						Violent convulsions, which ceased after 3:00 P.M.
Nov.						
1	102.	84	7.2	11	442	250 g. meat, 100 g. bread, 250 c.c. milk
2	102	66	7.7	12	349	Food as above

TABLE NO. 4—(Continued)

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
<b>1921</b>						
Nov.						
3	101	60	7.7	18	327	Food as above
4	101	60	7.8	16	347	" " "
5	101	62	8.1	16	388	" " "
6	100.8	62	8.2	12	393	" " "
7	100.8	64	8.4	12	395	" " "
8	100	70	8.7	14	492	" " "
9	101	68	8.9	12	471	" " "
10	101.2	76	9	14	494	" " "
11	101	80	9.2	14	520	" " "
12	102	80	9.3	16	579	Check test 583.
13	101	66	9.8	16	434	Very drowsy.
14	101.2	78	10	16	499	" " "
15	101	74	10.1	16	485	" " "
16	101.2	64	10.1	16	504	" " "
17	101	62	10.2	16	469	" " "
18	101	60	10.4	16	457	" " "
19	101	66	10.4	16	507	" " "
20	101	62	10.5	14	427	" " "
21	100.5	62	10.6	16	448	" " "
22	101	70	10.9	16	527	" " "
23	101	80	11	18	630	" " "
24	101	76	11.1	20	535	" " "
25	101	76	11.2	16	490	" " "
26	101	80	11.2	16	524	" " "
27	100	76	11.3	18	499	" " "
28	101	60	11.3	20	477	" " "
29	101	64	11.4	12	459	" " "
30	101	68	11.4	14	500	" " "
Dec.						
1	101	66	11.4	14	463	" " "
2	102	70	11.6	14	546	" " "
3	102	80	11.6	16	560	" " "
4	100.5	74	11.6	18	497	" " "
5	101	72	11.8	18	473	" " "
6	101	64	11.8	14	503	" " "
7	100.5	66	11.8	16	501	" " "
8	100	68	11.8	16	500	Check test 505.
9	100	62	11.9	16	491	" " "
10	101.2	72	12	16	500	" " "
11	101	62	12.1	14	491	Very drowsy.
12	101	64	12.1	14	497	" " "
13	100.2	70	12.2	16	494	" " "
14	100.2	70	12.2	18	493	" " "
15	101.5	72	12.2	14	522	" " "
16	101	60	12.2	14	502	" " "
17	101	65	12.2	14	499	" " "
18	100	62	12.2	16	490	" " "
19	100	62	12.3	14	498	" " "
20	100	66	12.2	16	528	" " "
21	100	68	12.2	16	516	" " "
22	100.4	62	12.2	16	500	" " "
23	100.4	68	12.2	16	510	" " "



TABLE NO. 4—(Continued)

Date 1992	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
Jan. 3	100.4	61	12.4	14	480	Body weight back to normal.
4	100.5	68	12.4	16	485	Food as above
5	100	70	12.5	20	500	" " "
6	101	68	12.5	14	508	" " "
7	100	60	12.5	16	445	" " "
8	100.5	66	12.5	20	446	" " "
9	100	64	12.5	14	456	" " "
10	100	64	12.6	16	437	" " "
11	101	80	12.8	20	508	" " "
12	101	70	12.8	16	499	200 g. meat, 100 g. bread, 250 c.c. milk
13	101	64	12.8	16	454	Food as above
14	101	72	12.8	16	576	" " "
15	100.8	64	12.8	18	481	" " "
16	101	64	12.8	16	498	" " "
18	101	72	12.8	14	504	" " "
19	100.8	64	12.8	16	504	" " "
20	100.8	70	12.8	18	510	" " "
21	100.1	72	12.8	14	565	" " "
22	100.8	76	12.7	16	418	" " "
23	100.1	72	12.6	16	477	" " "
24	101	60	12.7	16	460	Check test 460.
25	100	60	12.7	14	424	" " "
26	100	66	12.7	18	466	" " "
27	100	60	12.7	18	464	" " "
28	100	58	12.7	16	462	" " "
29	100.4	52	12.6	18	501	" " "
30	101	68	12.7	18	555	" " "
31	101	70	12.7	16	603	Check test 611.
Feb. 1	100.5	56	12.7	20	491	" " "
2	101	56	12.6	20	485	Check test 497.
3	100	58	12.7	16	468	" " "
4	100	64	12.6	18	510	" " "
6	100	64	12.7	14	524	" " "
9	100	50	12.8	16	580	" " "
10	101	64	12.8	16	514	Check test 530. 200 g. meat, 50g. bread, 250 c.c. milk.
11	101.5	68	12.7	14	583	Food as above
12	100.2	74	13.8	14	545	Check test 543.
13	101	74	12.8	18	524	Check test 519.
14	101	72	12.7	16	563	" " "
15	101	72	12.7	16	610	" " "
16	101.1	76	12.7	18	540	" " "
17	101.2	76	12.7	20	546	" " "
18	100.8	76	12.7	20	538	" " "
19	100.8	78	12.7	22	606	" " "
20	100.8	78	12.7	16	627	" " "
21						Mechanical difficulties.
22	100.2	72	12.7	22	529	" " "
23	100.2	74	12.8	14	620	" " "
24	100.2	68	12.7	14	544	Check test 531.
25	100.2	80	12.7	14	577	" " "
28	100.8	76	12.8	14	535	" " "



TABLE NO. 4—(Continued)

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
<b>1922</b>						
<b>Mar.</b>						
3	100.8	72	12.7	14	485	Food as above
4	101	60	12.8	14	493	" " "
5	101	60	12.7	18	494	" " "
6	101	64	12.8	22	478	Check test 490. " " "
8	101	68	12.8	18	476	" " "
9	100.8	60	12.8	18	455	" " "
10	100.8	54	12.9	18	484	" " "
11	100.8	60	13	22	526	" " "
12	100	84	12.8	22	568	Check test 563. " " "
13	100	60	12.8	14	478	" " "
14	100.8	70	12.9	16	569	" " "
15	100	64	12.9	14	475	" " "
16	100	64	12.9	14	529	" " "
17	100	58	12.9	14	512	" " "
18	100.2	85	12.8	14	617	Check test 605. " " "
19	100	64	12.9	16	479	" " "
20	100	66	12.9	14	561	" " "
21	100	64	12.7	14	509	" " "
22	100.2	62	12.8	14	516	" " "
23	100.2	72	12.8	16	536	" " "
24	100	64	12.9	14	526	" " "
25	100	68	12.9	14	558	" " "
26	100	64	12.9	16	518	" " "
27	100	68	12.9	14	493	" " "
28	100.2	68	13	18	573	" " "
29	101.3	68	13	22	462	" " "
30	101.3	68	12.9	16	496	" " "
31	101.3	60	13	14	503	Check test 511. " " "
<b>Apr.</b>						
1	100.8	72	13	14	490	" " "
2	101.3	70	13	14	510	" " "
5	100.6	72	13	18	540	" " "
6	100.6	64	13	18	560	" " "

period. The heat production per kg. of body weight on the last day of the fast was 42.4 calories per kg. of body weight. There was a slight but constant decrease in the body temperature of the dog during the last 17 days. The pulse rate decreased somewhat irregularly throughout the fast. The lowest pulse was 40 per minute.

*The Period after Fasting (December 23rd to May 5th).* No symptoms of gastric or other disturbed conditions were caused by breaking the fast. On December 23rd the dog was fed 50 g. of uncooked meat, 100 g. of bread, and 375 cc. of milk. The basal metabolism the next morning was 45.9% higher than what it had

been on the previous day. The dog was probably not in the post-absorptive condition, since only 12 hours had passed since feeding. This is the only one of the subjects that showed a marked increase in the basal metabolism the first day after eating. It was also the only subject that received and retained uncooked meat on the day that the fast was broken. Experimentation on this dog was interrupted from December 24th to January 3rd. During these days she received the diet of the stock animals in the laboratory. On and after January 3rd the animal received the maintenance diet of the control period until its initial body weight was reached and the experimental conditions were comparable to those of the control period in every possible way.

*The Basal Metabolism.* The average daily heat production for the entire after fasting period (January 3rd to May 5th) was 535.6 calories. The average body weight was 10.8 kilos. The average heat production per 24 hours per average kg. of body weight was 49.6 calories, an increase of 13.28% above the average control metabolism. The after fasting period of this dog, as in case of Dog No. 1, is best interpreted by subdividing the period so that the effect of the metabolism during the rapid increase in body weight and the estrual cycle can be separately presented. The average number of calories of heat produced from January 3rd to March 28th (body weight normal) was 554. The average body weight was 10.1 kilos. The average number of calories of heat produced per average kg. of body weight was 54.8, an increase of 27.7% above the average normal control metabolism. The average body weight of this dog from March 28th to May 5th was 12.39 kilos. The average daily heat production was 496.4. The average heat production per average kg. of body weight was 40.3 calories, 6% below the control.

*The Basal Metabolism during the Estrual Season (April 18th to May 5th).* The average heat production for this period of 18 days was 492.5 calories. The average body weight was 12.5 kilos. The average number of calories of heat produced per average kg. of body weight was 39.4. The average basal metabolism for a period of 18 days immediately preceding this rut was 502.1 calories. The average body weight was 12.3 kilos. The average number of calories per average kg. of body weight per 24 hours was 40.8. The basal metabolism during the rut was 3.4% lower than the basal metabolic rate for an equal number of days immediately preceding the rut.

TABLE NO. 5  
Daily Data for Subject Dog No. III

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
<b>1921</b>	<b>A. Control Period</b>					
<b>Oct.</b>						
17	101	74	12.2	16	532	250 g. meat, 100 g. bread, 250 c.c. milk
18	101	74	12.2	16	526	Food as above
19	101	66	12.1	16	528	" " "
20	101	78	12.2	14	528	" " "
21	101	66	12.2	14	503	" " "
22	101	76	12.3	16	512	" " "
23	101	78	12.2	16	513	" " "
24						Mechanical difficulties.
25						Mechanical difficulties.
26	100	66	12.2	12	544	" " "
27	101	74	12.2	16	528	" " "
28	100	66	12.2	16	501	" " "
29	100	74	12.2	14	472	" " "
30	100	76	12.1	12	533	" " "
31	101	76	12.1	16	519	Check test 522. " " "
<b>Nov.</b>						
1	101	76	12.1	12	526	" " "
2	101	76	12.1	16	503	" " "
3	101	76	12	16	490	Check test 490. " " "
4	101	76	12	16	491	" " "
5	101	74	12.1	16	491	" " "
6						Data discarded due to shivering.
7	101	78	12	16	517	Food as above
8	101	76	11.8	16	525	" " "
9	101	72	11.8	16	490	" " "
10	101	60	12	16	538	" " "
11	101	66	12	16	529	" " "
12	101	66	12	16	518	Last food at 8:00 A.M.
	<b>B. Fasting Period</b>					
13	101	66	12	16	509	1st Fast Day
14	100.8	66	11.8	16	527	Check test 527. 2nd " "
15	100.1	60	11.3	16	516	3rd " "
16	100.5	63	11.2	16	491	4th " "
17	101	60	11.1	18	477	5th " "
18	101	60	10.7	16	523	6th " "
19	101	60	10.5	16	430	7th " "
20	101	60	10.3	18	440	8th " "
21	100.8	62	10.2	18	476	9th " "
22	100.9	64	10	16	422	10th " "
23	100.9	64	10	16	461	11th " "
24	101	64	9.9	14	427	12th " "
25	101	62	9.7	14	464	13th " "
26	101	62	9.4	12	406	14th " "
27	101	62	9.2	12	395	15th " "
28	100	60	9.2	12	355	16th " "
29	99.5	54	9	12	349	17th " "
30	100	54	8.9	10	389	18th " "
<b>Dec.</b>						
1	100.8	50	8.8	10	351	19th " "

TABLE NO. 5—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
<b>1921</b>	<b>B; Fast ing Period — Continued</b>					
Dec. 2	100.8	52	8.7	8	361	20th " "
3	101	48	8.6	8	339	21st " "
4	101	60	8.4	8	385	22nd " "
5	101	48	8.3	10	314	23rd " "
6	100.8	46	8.2	8	330	24th " "
7	99.8	48	8.1	7	335	25th " "
8	100	48	8	7	349	26th " "
9	100	48	8	8	356	27th " "
10	100	48	7.9	8	362	28th " "
11	100	48	7.7	7	337	29th " "
12	100	40	7.6	7	352	30th " "
13	99.6	46	7.4	7	329	31st " "
14	99.6	40	7.2	7	305	32nd " "
15	99.6	44	7	6	314	33rd " "
16	99.4	42	6.9	6	318	34th " "
17	99.6	40	6.8	7	300	35th " "
18	99.4	40	6.8	8	314	36th " "
19	99.4	40	6.7	7	284	37th " "
20	100	44	6.7	6	310	38th " "
21	99.4	46	6.7	6	294	39th " "
22	99.4	46	6.6	6	281	40th " "
23	99.4	46	6.6	6	280	30 g. meat, 10 Og. bread, 1 pint milk at 5:00 P.M.
<b>24</b>	<b>C. Period After Fasting</b>					
<b>1922</b>	99.6	52	6.9	10	408*	50 g. meat, 1 pt. milk, 100 g. bread
Jan. 3	101	68	7.4	12	331	250 g. meat, 100 g. bread, 250 c.c. milk
4	101	68	7.4	12	371	Food as above
5	101	60	7.5	14	381	" " "
6	101	76	7.6	12	432	" " "
7	99.6	60	8	12	403	" " "
8	102	62	8.1	14	460	" " "
9	101	62	8.1	12	494	" " "
10	101	80	8.2	12	447	" " "
11	101	66	8.6	14	434	" " "
12	101	66	8.6	16	474	" " "
13	101	66	8.7	12	517	" " "
14	100.8	66	8.7	14	461	" " "
15	101	70	8.8	12	487	" " "
16	100.8	70	8.8	12	566	" " "
17	101	68	8.8	16	478	" " "
18	101	66	8.8	12	407	" " "
19	101	72	8.9	12	513	" " "
20	102	80	8.9	12	502	" " "
21	101	76	8.9	12	517	" " "
22	100.6	70	9	12	527	" " "
23	100.6	70	9	16	452	" " "
24	100	74	9.1	16	496	Check test 496. " " "
25	100.8	68	9.2	16	476	" " "
26	100.6	72	9.4	14	554	" " "

\*Dog was evidently not in the post-absorptive condition.

TABLE NO. 5—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
1922	C. Period	After	Fasting—	Continued		
Jan. 27	100	64	9.6	16	510	250 g. meat, 100 g. bread, 250 c.c. milk
28	100.2	82	9.8	16	510	Food as above
29	100	80	9.9	12	540	" " "
30	99.8	70	10	14	649	Check test 649.6. " " "
31	99.8	68	10.1	16	653	" " "
Feb. 1	99.8	76	10.2	16	524	" " "
2	100.4	62	10.3	14	606	" " "
3	100.4	80	10.3	16	568	" " "
4	100.4	68	10.3	16	556	" " "
5						Mechanical difficulties.
6	99.8	70	10.2	18	554	" " "
7	100	76	10.2	14	581	" " "
8	100.2	76	10.1	16	584	Check test 580. " " "
9	100	72	10	16	631	" " "
10	100.2	70	10	16	626	" " "
11	100.4	62	10.1	16	519	" " "
12	101	86	10	16	604	" " "
13	100	80	10.2	18	632	" " "
14	101.5	82	10.3	14	540	Check test 530. " " "
15	100.8	82	10.3	16	580	" " "
16	100.8	86	10.3	16	578	" " "
17	100	86	10.4	16	582	Check test 574. " " "
19	99.8	86	10.5	16	600	" " "
20	99.8	88	10.5	16	602	" " "
21	100	86	10.6	14	684	Check test 700. " " "
22	99.8	80	10.7	16	588	" " "
23	99.8	86	10.7	18	631	Check test 625. " " "
24	99.8	80	10.8	14	610	Check test 602. " " "
25	100	80	10.8	12	710	" " "
27	100	68	10.8	16	732	Check test 742. " " "
28	100	68	10.9	16	713	" " "
Mar. 1	99.8	70	10.9	16	655	" " "
3	99.8	72	11	14	550	" " "
4	99.8	70	11.1	16	525	" " "
5	100.2	68	11.1	14	570	Check test 582. " " "
6	100	84	11.2	14	699	Check test 687. " " "
7	99.8	84	11.1	14	605	" " "
8	100	68	11.3	16	559	Check test 564. " " "
9	100	82	11.4	16	651	Check test 651. " " "
10	100	64	11.5	16	551	Check test 551. " " "
11	99.8	60	11.6	18	665	" " "
12	100	78	11.6	18	629	" " "
13	99.8	80	11.7	16	673	" " "
14	99.8	80	11.8	18	536	" " "
15	100	88	11.8	18	614	" " "
16	100	78	11.8	18	627	Check test 636. " " "
17	99.8	60	11.8	18	583	" " "
18	99.8	60	11.7	18	524	" " "
19	100.6	80	11.5	18	569	" " "



TABLE NO. 5—Continued

Date	Temp. in F°	Pulse Rate per Min.	Wt. in Kilos	Resp. per Min.	Cal. per 24 Hrs.	Remarks
<b>1922</b>	<b>C. Period After Fasting—Continued</b>					
Mar.						
20	100.6	60	11.6	18	578	250 g. meat, 100 g. bread, 250 c.c. milk
23	100	64	11.7	16	513	Food as above
24	100.2	80	11.8	18	514	" " "
25	99.8	68	11.8	14	514	" " "
26	99.8	60	11.9	16	597	" " "
27	100	60	11.9	18	598	" " "
28	99.0	60	12	18	484	" " "
29	100	68	12.1	18	464	" " "
30	100	60	12.1	18	483	" " "
31	100	64	12.2	16	453	" " "
Apr.						
1	99.8	80	12.2	20	443	" " "
2	100	60	12.2	16	476	" " "
4	100.8	60	12.2	20	464	" " "
5	100.8	60	12.3	20	471	200 g. meat, 50 g. bread, 250 c.c. milk
6	100	68	12.4	20	471	Food as above
7	99.8	64	12.1	20	522	" " "
8	99.8	64	12.3	20	528	" " "
9	100	58	12.3	16	466	" " "
10	100	54	12.3	16	453	" " "
11	99.8	68	12.3	16	507	" " "
12	100	72	12.3	20	616	" " "
13	100	68	12.3	18	571	" " "
14	100	64	12.3	18	568	" " "
15	100	64	12.4	18	478	" " "
16	99.8	64	12.3	16	500	" " "
17	99.8	64	12.4	16	568	" " "
18	100.2	60	12.4	14	491	Indications of Rut.
19	99.8	64	12.4	14	517	" " "
20	100	60	12.4	12	466	" " "
21	101	64	12.4	16	482	" " "
22	101	80	12.5	18	516	" " "
23	101	72	12.4	18	516	Check test 517.
24	99.8	68	12.5	16	527	Check test 518.
25	99.8	64	12.6	22	460	" " "
26	99.8	72	12.6	16	483	" " "
27	100	68	12.6	16	572	" " "
28	100	68	12.6	20	469	" " "
29	99.8	68	12.6	16	443	" " "
30	99.8	68	12.6	16	445	" " "
May						
1	100	64	12.6	20	467	" " "
2	99.8	64	12.5	16	461	" " "
3	99.8	68	12.6	16	477	" " "
4	99.8	68	12.6	16	589	" " "
5	100.2	68	12.6	16	485	" " "



*Discussion of Results.*

*The Control Period.* Daily variations occurred in the basal metabolism of all subjects. The variations were greater for the period after fasting than for the pre-fasting period. The greatest deviation from the average for any subject during the control period was an increase of 13.6% and a decrease of 9.8%. The daily determinations for each subject can be found in Tables 1, 2, 3, 4 and 5. If the metabolism of any subject per unit of body weight be compared month by month during the same season of the control period, a striking uniformity in the results becomes apparent. Thus in the case of subject M. K., the average daily heat production per kg. of body weight for the month of July was 24.73 calories, for August 24.76 calories, in spite of the fact that daily variations during some of this time were exceedingly great. On August 29th the total heat production was 1267 calories. On August 13th the total number of calories for 24 hours reached 1476. Similar results can be found in the data of the dogs.

In many instances the increased metabolic rate was accompanied by an increase in pulse rate or a very slight increase in temperature but the numerous exceptions to this gives further evidence of the fallacy in attempting to apply mathematical precision to such functional relationships as pulse rate and intracellular oxidation.

The data presented in Tables 1, 2, 3, 4 and 5 proves conclusively that a single basal metabolism determination (using short periods and indirect methods) is unreliable and cannot be used for accurate comparisons in research work. However, there are many times when daily differences were less than 1%, but variations of 6% to 12% are of frequent occurrences in the basal metabolic rates of both man and dog.

The control period for dog No. 1 was much shorter than for either of the other dogs. The basal metabolism for this dog appears to be remarkably constant but this is apparent rather than real. The reason for the discrepancy is that tests which showed marked variation were discarded, on the assumption that the technique was faulty and that there is no variation in a normal dog's basal metabolism. It was later found that when all experimental factors were controlled, the dog motionless, and 18 hours after feeding, variations in the metabolism still occurred occasionally, if tests were carried on over a period of time, using this indirect method and 15 minute periods.

The respiratory rate seemed to have but little effect on the total heat production. The marked changes in the respiratory rate in case of the dogs occurred not because of appreciable changes in the room temperature but during sultry mornings and seemed to be due to an increase in humidity.

*The Fasting Period.* The ability to withstand the fasting varied with the several subjects. F. H. continued his daily occupation in the flour mill and since he has experienced previous fasting periods of even longer duration than this, he did not seem to be at all disturbed by the starvation except for the relatively small loss in body weight and a diminution of the pulse rate which occurred on the 15th fast day. The small loss in body weight of the subject can probably best be explained on the basis of the experiments of Howe and Hawk<sup>16</sup> on "repeated fastings." They conclude that a repeated fast is accompanied by less nitrogen loss from the body than from an original fast.

The subject M. K. also continued her daily occupation as described under methods. On the morning of the second, third and fourth fasting days there was a feeling of weakness and a tendency to perspire freely on the least exertion. The buccal mucous membrane became very dry after the first few days. This dryness neither created thirst nor seemed to be relieved by drinking water, in fact the desire for water was almost nil. If an increase in the water intake of a fasting organism stimulates nitrogen catabolism, it would seem to follow that this lack of thirst is a mechanism for conserving the forces of the starving body. Enemas were given on the third, fourth, sixth and eleventh fasting days. Spontaneous bowel movements occurred on the 12th and 13th days of the fast. Menstruation began on the 12th day (a few days in advance of the normal cycle) and seemed normal in every way. The muscular weakness was exceedingly great on and after the 13th day. On the 14th day there was some nausea accompanied by marked general weakness and distress. At the end of the 15th day, the muscular weakness was such that climbing four flights of stairs at 5 P. M. seemed almost impossible; in fact, the subject was obliged to rest at the top of each flight. There was no time during the 15 days of fasting when the subject felt entirely free from hunger. No change occurred in the pulse rate or body temperature during the fast.

Individual variations occurred in the reaction of the three dogs to the starvation. Dog No. 1 was depressed during much of

the time. It vomited occasionally and showed other symptoms of disturbance that may have been caused by the cooked bones fed to it. On the 37th day it was in a moribund state, refused food, and vomiting followed each attempt at forced feeding. On the 3rd day after fasting, the vomiting ceased. The dog displayed no further symptoms of functional disturbances.

Dog No. 2 received smaller portions of bone and endured the fasting much better. However, on the 40th fast day, the animal's general condition was very wretched. It refused food, could not stand alone and displayed symptoms of central nervous system disturbances. When forced to its feet it walked in a circle. Nystagmus was present. The dog was unable to retain nourishment for the first 3 days after the fasting. On the 2nd day it was seized with violent convulsions which seemed to be aggravated by the sensory stimulation involved in caring for it. During these convulsive seizures, the dog's head was retracted and the four legs extended. Vomiting ceased on the 3rd day after fasting, the convulsions on the 4th day; no further disturbances were observed.

Dog No. 3 (the older animal) gave no signs of functional disturbance during the 41 days of starvation. The body became extremely emaciated. The pulse rate diminished and there was a slight lowering of the rectal temperature. No gastric disturbance or other symptoms of distress were observed as a result of breaking the fast.

Cooked bones were fed to dogs No. 1 and No. 2 from time to time during the starvation period, on the assumption that they contained no food value but would serve to keep the animals more content and in a better physiological state by stimulating bowel movements. But the effect of feeding the bones to fasting dogs seems deleterious rather than helpful, since dogs No. 1 and No. 2 received bones and were markedly disturbed while dog No. 3 received no bones, fasted longer and showed no functional disturbances either during or on breaking the fast.

*The Basal Metabolism during Fasting.* The most striking feature of the fasting metabolism of these five subjects was the marked increase in the basal metabolic rate which occurred during the first part of the fasting period of the two people usually apparent on the 2nd or 3rd fasting day. The metabolic rate for the entire 15 fast days for M. K. was higher than normal. For F. H. it approached the normal level on the 9th day but never fell

below that of the lowest normal, even on the 15th day of starvation.

Dogs No. 1 and No. 2 maintained a higher metabolic rate than normal while the basal metabolism of dog No. 3 remained remarkably constant throughout the entire 41 days of starvation (excepting the slight rise on the 6th day). These studies on fasting metabolism do not conform to the conclusions drawn by Benedict<sup>18</sup> on the basis of his experiments on fasting people, i.e., that the first period of a fast is characterized by a rapidly falling metabolism followed by a period of level metabolism and finally a tendency toward an increase in the basal metabolic rate. It should be noted in this connection that in converting the cc. of  $O_2$  consumed into calories of heat, I have assumed that the respiratory quotient was .82. This, of course, is not true for the fasting period since the respiratory quotient for all subjects was probably near .71 during the fasting period. This would change the calorific value of the  $O_2$  consumed so that the total number of calories of heat computed for each subject during this period is about 2% too high. The high fasting metabolism of dogs No. 1 and 2 is no doubt due to their unusually disturbed conditions.

#### *Period after Fasting.*

*Body Weight of the Two Persons.* The period after fasting for M. K. was of six months' duration. The ingestion of the first food after fasting is very interesting in that the physiological effects can scarcely be explained on the basis of the caloric value of the food eaten. On September 28th (last fast day) at 5:00 P. M. the general feeling of weakness and distress was very great as noted above. At 6:00 P. M. an orange (about 100 calories) was eaten. In a very short while the feeling of great weakness and distress had given place to a feeling of normal muscular strength and well-being. At 6:30 a bullion cup of tomato soup (200 calories) was eaten. Shortly after this the subject descended four flights of stairs, walked about in the neighboring park for an hour, returned and climbed the four flights of stairs without the least feeling of fatigue. Another bullion cup of tomato soup was eaten before retiring. The basal metabolism was 2.5% lower the next morning than it had been at any time during the fasting period. The pulse rate was normal.

The increase in body weight following starvation was amazingly rapid. By the end of the 7th day after fasting the body



had gained 17.38 lbs., the weight was back to normal in spite of the fact that during the first five of these days only one moderate meal a day had been eaten. It seems that the mechanism by which the cells of an extremely emaciated body, rendered thus by starvation but organically sound, utilizes food, must be quite different from that which occurs after emaciation from sickness, when not only body substance must be built up, but functional disturbances corrected as well. Certainly the body is not able to utilize food on such an economic basis under ordinary conditions of nutrition. After the body weight had returned to normal (55.1 kilos.), the increase continued slowly until a weight of 57.5 kilos. had been reached. Following this there were variations from 57.5—57.2 throughout the entire period. The actual gain in body weight over the control period was 1.8 kilos.

No changes were detected in the temperature or the pulse. The appetite was unusually keen for about two months after the fast had ended. The bowel movements were more regular for at least two months after the fast.

The body weight of the subject F. H. increased 18.2 lbs. during the first week after fasting (December 3-11). At the end of the period (February 14) the weight was 65 kilos., an increase of 7 lbs. over the initial weight. The pulse rate of this subject showed a slight increase as a result of the fasting.

The feeling of well-being and apparent increase in energy experienced by these two people conform to the results described by Carlson<sup>19</sup> after a five-day fast.

It is difficult to attempt a discussion of the rapid increase in body weight of the two persons, since quantitative determinations of the dietary were not made. It is possible that the fasting caused a hydration and that part of the rapid increase in weight which occurred during the first 3 or 4 days, was due to an accumulation of fluids in the tissues rather than to an increase in body substance. But if this did occur, the fluids must have been rapidly replaced by active protoplasm, since the gain in weight was permanent and the increase in the amount of oxygen consumed after the first 4 or 5 days could not have been caused by inert water.

*Body Weight of the Three Dogs.* The time required for the dogs to recover their respective body weights of the control periods varied from 4 to 11 weeks (Tables 3-4-5). There was no astonishingly rapid increase in weight during the first days after

fasting as occurred with the two persons, but a uniformly progressive gain. Each dog daily received the carefully weighed maintenance diet of the control period until its initial body weight had been reached. Following this, the amount of food given each dog was gradually diminished in an effort to keep the body weight at the same constant level of the control period. The total reduction of the maintenance diet was such that the final dietary of each dog for this period had a fuel value of 643 calories, 218 calories less than the maintenance diet of the control period. In spite of this marked reduction in the amount of food ingested, the body weight of each dog was greater when experimentation ceased than it was during the control period.

Von Seeland<sup>20</sup> found that pigeons starved for a period of 15 days and subsequently fed attained a greater body weight than those of the same hatching that had not been starved. Noe<sup>21</sup> has observed a similar condition in regard to rats, mice, guinea pigs and rabbits. Dietrick<sup>22</sup> found that swine which had been starved for ten days and subsequently fed could be maintained in a condition of nitrogenous equilibrium on a diet much less than that which they received during the prefasting period.

These results seem to indicate that fasting, at least in some higher animals, causes a change in the ability of the cells to assimilate food. It seems that the body, reduced to its own resources, acquires an economy which changes the nutritive value of the food relative to a starved and an unstarved cell. The underlying principles of nutrition involved in such a process must be found in the hidden recesses of the living cell and cannot be deduced from a study of caloric values or chemical composition of foods. If these results signify an increase in the nutrition of the body, the effect of fasting should be investigated in connection with wasting diseases and diseases characterized by lowered nutritional conditions.

*Basal Metabolic Rate after Fasting.* The basal metabolic rate of both persons was lower on the day after fasting than it had been at any time during the fast. Subject M. K. remained low for the first 4 days of this period. Determination on dogs No. 1 and No. 2 were discarded because of their disturbed conditions during the first 4 days. Dog No. 3 showed a marked increase in the basal metabolic rate on the day after fasting probably due to retarded digestion and absorption and consequently prolonged specific dynamic effects of the meat eaten the previous day. Follow-



TABLE 6

Average results of the control, fasting and after-fasting periods, and the percentage deviation from the control metabolism.

Subject	Period Date Number of Days Number of Determinations	Average Number of Calories Produced per 24 Hrs.	Average Body Weight in Kilos	Average Number of Calories per Average Body Weight per 24 Hrs.	Percent Deviation from Control Metabolism
M. K.	Control July 1-Sept. 14 75 days 74 determinations	1354.8	55.1	24.58	
M. K.	Fasting Sept. 14-28 15 days 15 determinations	1403.2	50.3	27.8	+12.6*
M. K.	After fasting Sept. 29-Oct. 4 5 days 5 determinations	1292	51.3	23.2	- 5.6
M. K.	After fasting Oct. 5-Dec. 4 62 days 61 determinations	1465	56.2	26.07	+ 6.1
M. K.	Six months after fasting Oct. 4-Mar. 31 179 days 161 determinations	1475.7	57.2	25.79	+ 4.5
F. H.	Control Nov. 3-17 15 days 8 determinations	1435.7	61.1	23.4	
F. H.	Fasting Nov. 19-Dec. 3 15 days 8 determinations	1403.9	58.4	24.02	+ 2.6*
F. H.	After fasting Dec. 4-5 2 days 1 determination	1235	58	21.3	- 8.9
F. H.	After fasting Dec. 7-Feb. 14 70 days 17 determinations	1526.3	63.7	23.9	+ 2.1

TABLE 6—*Continued*

Subject	Period Date Number of Days Number of Determinations	Average Number of Calories Produced per 24 Hrs.	Average Body Weight in Kilos	Average Number of Calories per Average Body Weight per 24 Hrs.	Percent Deviation from Control Metabolism
Dog 1	Control Mar. 21–Apr. 9 19 days 7 determinations	360 1	10 2	35 3	
Dog 1	Fasting Apr. 9–May 16 37 days 36 determinations	305.3	8 04	37.9	+ 7.3*
Dog 1	After fasting May 19–June 19 32 days 32 determinations	383 4	9 1	42 1	+19.3
Dog 1	After fasting June 19–Aug. 2 44 days 43 determinations	407 5	10 2	39.9	+13.0
Dog 1	After fasting Aug. 28–Oct. 29 62 days 61 determinations	280	10 5	36 2	+ 2 2
Dog 2	Control July 9–Sept. 16 50 days 42 determinations	465.3	12 4	37.54	
Dog 2	Fasting Sept. 17–Oct. 27 40 days 39 determinations	391 8	9 78	40	+ 6 7*
Dog 2	After fasting Nov. 1–Jan. 4 65 days 55 determinations	483 7	10 7	45 2	+20.4
Dog 2	After fasting Jan. 4–Apr. 6 92 days 80 determinations	515 6	19 9	39 98	+ 6.5
Dog 3	Control Oct. 17–Nov. 12 27 days 24 determinations	515.5	12	42 9	

\*Values are 2-3% high due to lowered respiratory quotient of fasting.

TABLE 6—*Continued*

Subject	Period Date Number of Days Number of Determinations	Average Number of Calories Produced per 24 Hrs.	Average Body Weight in Kilos	Average Number of Calories per Average Body Weight per 24 Hrs.	Percent Deviation from Control Metabolism
Dog 3	Fasting Nov. 13-Dec. 23 41 days 41 determinations	379.4	8.73	43.45	+ 1.2*
Dog 3	After fasting Jan. 3-Mar. 28 75 days 69 determinations	554	10.1	54.8	+27.7
Dog 3	After fasting Mar. 28-May 5 39 days 34 determinations	496.4	12.39	40.3	- 6

ing this all subjects showed a marked increase in the oxygen consumption per unit of body weight which was apparent for at least two months. After this there was a tendency to return to normal as can be seen from Table 6, but the exact level of the normal was never reached for the subjects M. K. and Dog No. 1.

TABLE NO. 7

Subject	Duration of Fast in Days	Percent Loss in Initial Body Weight	Percent Increase in B. M. R. 1-3 Months After Fasting
Dog No. 1.....	37	39.3	+19.3
Dog No. 2.....	40	42	+20.4
Dog No. 3.....	41	45	+27.7
M. K.....	15	14.3	+ 6.1
F. H.....	15	10.2	+ 3

The increased oxygen consumption after fasting cannot be attributed to an increase in the caloric intake of food because the dietary of the dogs for about two months after starvation was quantitatively the same as the maintenance diet of the control period. Voit's principle states that the intensity of metabolism in the cell is modified by the kind and amount of food brought to the cell

by the blood. It is conceivable that starvation causes a change in the permeability of the cell membranes in such a way that more food is able to reach the cell after starvation than before. This would result in an augmentation of the metabolism of the cell by the presence of increased quantities of food stuffs. If the cell membranes of young cells are more permeable to food stuffs than the membranes of older cells, the increase in metabolism which is characteristic of the young may be explained on Voit's theory. Child terms the increased oxygen consumption which occurs in starving planaria a process of "rejuvenescence." Allen<sup>23</sup> points out that after fission in a worm, the tail piece which becomes organized into a smaller sized worm shows a higher rate of oxygen consumption per unit of body weight. Hyman<sup>24</sup> finds a similar condition accompanying the regeneration of worms that have been cut into small pieces. DuBois has found that the basal metabolism of convalescents after typhoid fever may reach an increase of 17% above the normal. DuBois<sup>25</sup> notes that this heightened metabolism "is reminiscent of the increased heat production occurring during the period of growth."

It becomes evident that where the initial weight was reduced by 45%, and subsequently restored by the normal diet, approximately one-half of the restored body is made up of new protoplasm. In this there is rejuvenescence. But the fact that there is a tendency for the metabolic rate to return to its former level points to internal co-ordinating processes that are not permanently altered by fasting. The establishment for a period of months of a metabolic level, somewhat higher than normal, is probably due to improved nutrition due to the replacement of new cells for less resistant ones that were used to supply the body during the fasting.

This increased heat production after fasting is no doubt analogous to the increased metabolic rate which occurs during the period of growth in the young. In the case of the dogs, the first two months after fasting represents the most rapid regeneration of body tissues. During this time the food was not increased but the  $O_2$  consumption was markedly increased. The increased oxygen consumption parallels the anabolic processes of transforming the energy of the food into living protoplasm. The  $O_2$  consumed during the anabolic processes of building up tissue protein does not seem to conform to the oxygen consumed during catabolic processes as explained by methods evolved from a study of "bomb calorimetry."

Carlson suggests that the higher metabolism after prolonged fasts may be due to a temporary excess activity of such glands as the thyroid and the gonads, glands that seem to have direct effects on the metabolic rate. It is well established that fasting induces marked atrophy of these glands. The recuperation of these glands, on resumption of eating, may carry them, for a while, beyond the level of activity normal for the age of the subject. This would in all probability lead to a higher basal metabolism. We have data indicating increased activity of the gastric glands<sup>27</sup> following prolonged fasts.

*Effect of the Estrual Cycle on the Basal Metabolism.* The exact time of the beginning of rut is difficult to determine, but with these dogs a few drops of bloody discharge from the vagina could be detected on the pad where they lay during the metabolism tests. Perhaps most bitches discharge a slight amount of bloody fluid during this period, but it may not be generally observed because of its small quantity and the habits of cleanliness that characterize dogs. The only time that the discharge was noticed was during the period of taking the basal metabolism. It should be recalled that during these periods the dogs lay motionless for at least one hour so that discharges that ordinarily would have been removed accumulated in noticeable quantities. From Table 8 it can be seen that there was no increase in the B. M. R. during rut, but in two of the periods there was a decrease of from 3.4-4% while the one period shows no change. This precaution should be mentioned in making metabolism determinations on dogs in heat; it was difficult to get Dog No. 1 perfectly quiet at the beginning of the rest period. She seemed unusually alert and ready to respond to the least movement about her. But once having become perfectly quiet and relaxed she remained in the same motionless condition previously mentioned.

After the rut had ceased (August 26) there was a marked lowering of the basal metabolism which persisted until about the 27th of October. After this the metabolism became high and remained so until the end of the second rut season. The highest being the ten days immediately preceding the second rut season. The average basal metabolism during the post-estrual cycle (August 28 to October 27) was 380 calories per day. The average body weight was 10.5 kgs. The average number of calories of heat produced per 24 hours per average kg. of body weight was 36.2. This is a decrease of 8.12% below the average metabolism for the



TABLE NO. 8

Dog	Period	Average Number of Calories per Kg. of Body Wght.	Percent Deviation of Metabolism During Rut from an Equal Number of Days Preceding Rut
No. 1	23 days preceding rut, July 12-Aug. 3, 1921.....	39.2	
No. 1	Rut—Aug. 4-26, 23 days.....	39.4	
No. 1	15 days preceding rut, Jan. 29-Feb. 12, 1922.....	44.5	
No. 1	Rut—Feb. 14-28, 15 days.....	42.7	-4%
No. 3	18 days preceding Mar. 30-Apr. 17.....	40.8	
No. 3	Rut—Apr. 18-May 5, 1922, 18 days....	39.4	-3.4%

estrual season. It is to be regretted that metabolism determinations were not continued for at least two more months to determine whether the post estrual drop would again occur.

These experiments suggest a lowering of the metabolism during the latter part of the estrual cycle, followed by a still lower rate for a considerable period after the rut, after which there is a rise which reaches its greatest level just before the appearance of the next rut. Since the estrual cycles here cited all occurred after fasting, the fluctuations described may have had little bearing on the condition of rut per se.

*Effect of Menstruation on the Basal Metabolism.* Comparisons of the average basal metabolism of the first four days of menstruation and the average metabolism of the four days preceding menstruation can be found in Table 9.

From the Table it can be readily seen that the deviation of the metabolism during the first four menstrual days from the preceding four days is not constant. This conforms to the results obtained by Blunt<sup>26</sup>. But the tendency seems to be towards a lowering of the basal metabolism during the first four days of menstruation.

*Seasonal Variation.* A slight tendency toward a seasonal variation can be seen in the metabolism of the Subject M. K. Determinations in this case were made over long periods of time before



TABLE NO. 9  
Average Computed from Table 1

First Four Days of Menstrual Periods	Average Number of Calories for Four Menstrual Days	Four Days Immediately Preceding Menstruation	Average Calories for Four Days Preceding Menstruation	Percent Variation Metabolism During First Four Menstrual Days and Four Pre- ceding Days
April 5-8	1455	April 1-4	1494	-3.4
May 5-8	1440	May 1-4	1428	+ .8
June 1-4	1366	May 28-31	1350	+1.1
June 29-2	1362	June 25-28	1438	-5
Aug. 3-6	1337	July 30-2	1367	-2
Aug. 30-2	1331	Aug. 26-29	1361	-2.2
Sept. 25-28	1309	Sept. 21-24	1344	-2.6
Oct. 31-3	1409	Oct. 27-30	1509	-7.2*
Nov. 28-1	1496	Nov. 24-27	1439	-4
Jan. 26-29	1471	Jan. 22-25	1337	+1.6
Feb. 24-27	1529	Feb. 20-23	1555	-2

\*First period after fasting.

and after the fasting. The metabolism of this subject during July and August was slightly lower than it was during any other month of the year.

TABLE NO. 10  
Seasonal Variation

Subject	Month	Average Heat Production per Day	Average Body Weight	Average Number of Calories per Average Kg. of Body Weight	Percent Deviation from April Metabolism
M. K.	April, 1921	1427	55.2	25.86	
M. K.	July, 1921	1365	55.2	24.73	-4.3
M. K.	August, 1921	1362	55.1	24.76	-4.2
M. K.	March, 1922	1499	57.3	26.16	1.16

The seasonal variations of the dogs cannot be accurately distinguished from the effects of fasting and the estrual cycle.

#### Summary.

1. A muzzle was perfected, and a method devised by means of which the gaseous respiratory exchange of dogs can be accurately determined from either a closed or an open respiratory system.

2. Daily variations appear in the basal metabolic rate of both man and dog. Average results over periods of 3 or 4 weeks are remarkably uniform per unit of body wt.

3. The pulse rate does not always parallel the basal metabolic rate and therefore is not a reliable index of the cellular metabolism.

4. The basal metabolic rate during the initial days of fasting in man may be much higher than normal. A fast of 41 days in dogs and 15 days in man causes no appreciable lowering of the basal metabolic rate during the fasting period.

5. Dogs, fasted until their initial body weights had been reduced 39-45%, not only regained their normal weights but gained weight on the same dietary which before fasting merely maintained their body weights constant. After the fast they maintained normal weight on a lower calory intake. This indicates a more economical use of the food. It is not due to decreased general activity.

6. There is a temporary increase in the basal metabolic rate as an effect of prolonged fasting. This temporary increase seems to be proportional to the duration of the fast and loss in body weight.

7. The increased oxygen consumption in dogs was most marked during the time that the body was undergoing rapid growth or gain in weight.

8. The basal metabolic rate of female dogs during rut is normal or slightly lower than normal.

9. The basal metabolic rate during the first four days of menstruation is usually slightly subnormal.

10. There are indications of seasonal variations in the basal metabolic rate of man.

I wish to thank Professor Carlson for having planned the problem and for helpful criticisms during the completion of the plans.

## BIBLIOGRAPHY

1. Luciani, L.: *Das Hungern*, Leipzig, 1890.  
 Noel Paton, & Stockman: *Proc. Roy. Soc., Edinburgh*, 16, 1890, 121.  
 Senator Lehman et al.: *Virchow's Arch. u. path. Anat. u. Physiol.*, 131, 1893, Supplementheft. 1.  
 Cathcart: *Biochemische Zeitschrift*, 6, 1907, 109.  
 Benedict: *A Study of Prolonged Fasting*, Carnegie Institution of Washington, Pub. 203, 1915.
2. Earle: *Arch. Int. Med.*, 14, 1914, 8.  
 Mühlmann. *Russische Literatur über die Pathologie des Hungerns*, *Centralbl. f. allg. Path.*, 10, 1899, 160.  
 Linboudrow: *Diss.*, 71, 1893, Russian. From the Lab. of Path. Anat., St. Petersburg.  
 Stoppenbrink: *Zeitschr. f. Wissenschaftliche Zoologie*, 79, 1905, 490.  
 Morgulis: *Arch. f. Entwicklung mechanik der Organismen*, 32, 1911, 169.  
 Wallengren: *Zeit. f. allg. Physiol.*, 1, 1902, 167.
3. Carlson: *Amer. Jour. Physiol.*, 33-34, 1914, 94.  
 Patterson: *Amer. Jour. Physiol.*, 33-34, 1914, 423.  
 Ibid.: 1915, 37-38, 316.
4. Child: *Scenescence and Rejuvenescence*, Chicago, 1915.
5. Hyman: *Amer. Jour. Physiol.*, 49-1, 1919, 377.
6. Allen: *Amer. Jour. Med. Sc.*, N. S., 150, 1915, 480.  
 Stillman: *Amer. Jour. Med. Sc.*, N. S., 151, 1916, 505.  
 Joslin et al.: *Boston Med. & Surg. Jour.*, 174, 1916, 371.
7. Harris & Benedict: *A Biometric Study of Basal Metabolism in Man*, Washington, 1919.
8. Benedict: *Boston Med. & Surg. Jour.*, 78, 1918, 667.
9. Benedict: *Jour. Biol. Chem.*, 20, 1915, 295.
10. Lusk.: *Jour. Biol. Chem.*, 20, 1915, 562.
11. Benedict et al.: *Amer. Jour. Physiol.*, 27, 1910-11, 383.  
 Ibid.: *Jour. Biol. Chem.*, 18, 1914, 139.
12. Zuntz & Schumberg: *Physiol. des Marsches*, Berlin, 1901.  
 Riche & Lusk: *Jour. Biol. Chem.*, 12, 1912, 357.
13. Meeh.: *Zeitschr. f. Biol.*, 15, 1879, 425.
14. DuBois & DuBois.: *Arch. Intern. Med.*, 17, 1916, 863.
15. Atwater: *Dept. of Agriculture*, Washington, D. C. Bull. No. 28.
16. Howe & Hawk: *Jour. Amer. Chem. Soc.*, 33, 1911, 215.

17. Howe, Matill & Hawk: *Jour. Biol. Chem.*, 10, 1911, 417.
18. Benedict: *A Study of Prolonged Fasting*, Washington, 1915, 372.
19. Carlson: *Control of Hunger in Health and Disease*, Chicago, 1916, 137.
20. von Seeland: *Biol. Central*, bl., 7, 1887, 145.
21. Noe: *Compt. Rend. Soc. Biol.*, 52, 1900, 755.
22. Dietrick: *Ill. Agri. Exp. Station*, Feb., 1912, July, 11, 1913, Bul. 154-165, 411.
23. Allen: *Amer. Jour. Physiol.*, 49, 1919, 420.
24. Hyman: *Amer. Jour. Physiol.*, 1, 1919, 67.
25. Coleman & DuBois: *Arch. Intern. Med.*, 15, 1915, 887.
26. Blunt: *Jour. Biol. Chem.*, 47, 1921, 69.
27. Carlson: *Amer. Jour. Physiol.*, 45, 1917-18, 130.  
Kunde: Unpublished data.



## STUDIES ON THE PHYSIOLOGICAL ACTION OF SACHARIN.\*

By

A. J. Carlson, C. J. Eldridge, H. P. Martin, and F. L. Foran.

(From the Hull Physiological Laboratory of the University of Chicago)

It is well established that saccharin is not an acute poison. Daily ingestion of moderate quantities of saccharin for several months by healthy persons seem to produce no demonstrable injury, except such as may follow the dislike for the substance due to the peculiar after taste; and there seems to be much individual variations in the reactions to this after taste, some persons being so strongly affected by it that they prefer the alternative of taking their foods unsweetened. In larger doses saccharin causes headaches, gastro-intestinal disturbances, and mental possibly cardiac depression. How saccharin produces these effects has not, so far as we know, been determined. It is usually held that, except for the apparently selective action of saccharin on the organs of taste in the mouth, the substance is physiologically inert, and is excreted unchanged in the urine.

The extensive substitution of saccharin for sugar in foods during the war, especially in Europe, brought the question of possible toxicity of this coal tar derivative again to the fore. It would seem obvious that this question cannot be settled by a few months' feeding tests on healthy young men. Such persons probably have the largest "factors of safety" in all the organ systems, so that saccharin would have to produce large impairments before organ deficiency appeared. If saccharin is to be used as a general substitute for sugars in food and drinks it will be ingested by people of all ages, and all degrees of physiological impairments, not for a few months but for the duration of the life of the individual. This aspect of the question, of obvious importance to public health, is not touched by the work and report on saccharin by the Referee Board.

It seemed therefore of importance to determine whether saccharin has any action on the body, except on the organs of taste,

\* This work was undertaken at the request of Dr. Carl L. Alsberg, Chief, Bureau of Chemistry, U. S. Dept. of Agriculture, and the expense of the work was defrayed, in part, by the Bureau of Chemistry. The work was completed six years ago.



irrespective whether such action on healthy individuals is pathologically significant.

The question of purity of commercial saccharine may also be raised, in view of the recent developments in chemistry showing the difficulty, if not practical impossibility, of securing pure substances, even in the case of relatively simple inorganic compounds. This difficulty is necessarily increased in the case of substances derived from such a complex mixture as coal tar, a mixture containing at least some distinctly toxic ingredients.

*I. The action of saccharin on peptic digestion in vitro.*

Herter reported that saccharin in small doses (up to .3 gram daily) caused no disturbance of any kind in normal persons, and larger doses (up to 1.5 gram daily) caused certain modifications of physiological functions which were ascribable to saccharin. These were (a) a serious distaste for the substance due to the persistent sweet-metallic taste in the mouth, and (b) an increase in the free hydrochloric acid of the gastric juice in some instances which made it highly probable that saccharin was responsible for this modification. Such a rise does not seem to be pathologically significant. (c) A tendency to digestive disturbance. An investigation of the influence of saccharin upon the peptic digestion of egg albumin in the presence of hydrochloric acid showed, according to Herter, that concentrations of saccharin such as might be used in sweetening had no material influence upon the activity of the peptic enzyme. Roger and Garnier, in 1907, showed that acid saccharin cannot activate pepsinogen, although it has some activating power. Salkowski concluded from testing the action of pepsin with moist blood fibrin and saccharin and measuring the peptone content of the digestion mixture that saccharin has no effect on gastric digestion. However, with hard boiled egg albumin finely minced and mixed, saccharin in 0.1% had no effect, while saccharin in 0.25% and stronger had marked inhibitory effects. The criterion here used was the visible reaction.

The same result was obtained if the quantity of peptonized protein was used as the criterion. Gans claimed that the inhibiting action of saccharin in large doses was due to the excess of insoluble saccharin carrying down the ferment with it. Salkowski believes, however, in a specific action of the drug on protein digestion. Pflugge found that saccharin noticeably delays the diges-

tion of egg albumin. Paul observed that fibrin with saccharin was scarcely attacked when that without saccharin was completely dissolved and he concluded from other experiments that it inhibits all ferment action. Schmitt concludes that saccharin inhibits peptic digestion less than sugar solution of equal sweetness. Jensen, using a digestion mixture prepared from hog's stomach, concludes that acid saccharin, even 0.04%, inhibits somewhat and larger amounts very much. Gans concludes that saccharin had no bad effect on peptic digestion. Brauardel, Peuchet and Ogier found that 0.2%-0.3% slows gastric digestion. Petschek and Zerner claimed that there was no inhibitory action if the saccharin were completely dissolved or neutralized. They also found that digestion proceeded equally well with and without saccharin up to 1%. Nencki using coagulated egg white and graded amounts of saccharin, found that saccharin inhibits in proportion to the amount present.

For the purpose of determining whether saccharin, either as the sodium salt or the pure acid saccharin interferes with the course of peptic digestion the following experiments were devised.

Tests were made upon pure human gastric juice taken from a man with a gastric fistula, and upon gastric juice collected from dogs with Pavlov accessory stomachs. The digestion mixtures in two series were made up with 5 cc. of the gastric juice—and in one series with 9 cc. of gastric juice. Using one tube as a control, suitable volumes of a 5% solution of the sodium salt of saccharin were added to the remaining five tubes used in the test, so that each tube contained respectively 0.05 gram, 0.025 gram, 0.01 gram, 0.005 gram, 0.001 gram saccharin. The volume was made up to six cubic centimeters in the last series. In one series water was added to make the volume constant, and in the other two series N/40 NaOH was added. To check up the acidity of the control with the tubes containing saccharin, one cubic centimeter of each digestion mixture was titrated against N/40 NaOH for free and total acidity, after the 24 hour digestion period. The rate of digestion was measured fairly accurately by the length of the column of coagulated egg white dissolved away from the end of glass tubes (Mett's tubes). Two Mett's tubes were placed in each digestion tube and the whole group placed in a thermostat at 37°C., for 24 hours. The Mett's tubes were made by drawing egg

white up into glass tubes of  $1\frac{1}{2}$  to 2 mm. bore, sealing the ends in a flame and boiling in water for ten minutes.

Since the amount of pepsin present is constant, each tube receiving the same amount of pure gastric juice, we are concerned only with the rate of digestion, which is measured by the length of the column of coagulated egg albumin digested.

TABLE 1

Inhibition of peptic digestion due to Saccharin (sodium salt) using human gastric juice. Average of three experiments.

	mm. Digestion	% Free Acidity	% Total Acidity
I 5 cc. juice + 1 cc. H <sub>2</sub> O.....	11.9	.2887	.3522
II 5 cc. juice + 1 cc. 5% saccharin solution...	4.25	.2297	.2705
III 5 cc. juice + $\frac{1}{2}$ cc. 5% + $\frac{1}{2}$ cc. H <sub>2</sub> O.....	4.4	.2781	.3250
IV 5 cc. juice + 0.2 cc. 5% + 0.8 cc. H <sub>2</sub> O....	7.56	.2857	.3433
V 5 cc. juice + .1 cc. 5% + .9 cc. H <sub>2</sub> O.....	9.78	.2857	.3463
VI 5 cc. juice + .02 5% + .98 cc. H <sub>2</sub> O. ....	10.9	.2857	.3463

TABLE 2

Inhibition of peptic digestion due to Saccharin, using gastric juice collected from Pavlov pouch of dog. Average of two experiments.

	mm. Digestion	% Free Acidity	% Total Acidity
I 5 cc. juice + 1 cc. N/40 NaOH.....	4.5	.2583	.3250
II 5 cc. juice + 1 cc. 5% saccharin solution...	1.1	.2427	.2643
III 5 cc. juice + .5 cc. 5% + .5 cc. N/40 NaOH	1.2	.2492	.3190
IV 5 cc. juice + 2 cc. 5% + .8 cc. N/40 NaOH	2.38	.2522	.3190
V 5 cc. juice + .1 cc. 5% + .9 cc. N/40 NaOH	3.5	.2522	.3220
VI 5 cc. juice + .02 cc. 5% + .98 cc. N/40 NaOH.....	4.07	.2553	.3220

TABLE 3

Inhibition of peptic digestion due to Saccharin, using human gastric juice. Average of three experiments.

	mm. Digestion	% Free Acidity	% Total Acidity
I 9 cc. juice + 1 cc. N/40 NaOH.....	12.37	.2781	.3372
II 9 cc. juice + 1 cc. 5% Saccharin solution..	3.55	.2644	.3145
III 9 cc. juice + .5cc. + .5 cc. 5% N/40 NaOH.....	5.10	.2781	.3281
IV 9 cc. juice + .2 cc. 5% + .8 cc. N/40 NaOH	8.92	.2781	.3372
V 9 cc. juice + .1 cc. 5% + .9 cc. N/40 NaOH	10.5	.2827	.3372
VI 9 cc. juice + .02 cc. 5% + .98 cc. N/40 NaOH.....	10.8	.2827	.3372

The tables above show that the digestion was slowest in the tubes with the highest percentage of saccharin and that even in the tubes with least saccharin the digestion is slowed somewhat. It is seen in all three tables that the acidity of the mixtures, especially those in which there was a small amount of saccharin, is not decreased enough below the control to account for the difference in length of column of egg white digested. In Tables 1 and 2 the percentage of saccharin put into the respective tubes is 0%; 0.5%; 0.25%; 0.1%; 0.05%; and 0.01%. These percentages do not represent the actual concentration of saccharin in the solution for the acid reaction caused, at least part of, the sodium salt to change over into the acid saccharin, and this was thrown down as a crystalline precipitate. Acid saccharin is only very slightly soluble in pure gastric juice. Similar experiments to the above were performed in which a solution of the acid saccharin was used instead of the sodium salt. The results were similar in every respect.

*11. The effect of saccharin in the mouth on the appetite secretion of gastric juice in man.*

Our laboratory was fortunate in having the services of a man with a gastric fistula and complete cicatricial stenosis of the esophagus. The subject, Mr. V., was in good health. A great deal of experimental work had been done on Mr. V. so that he was quite used to the procedure of withdrawing the juice from his stomach. The experiments were run on days when he was not too busy with his regular work and when he felt in good physical condition. They were started at 10:00 in the forenoon, by introducing 200 cc. of water into his stomach through the fistula. This was for the purpose of washing out the stomach, and by eleven o'clock no water or food remained. At eleven o'clock he drained out the gastric residuum and any water that remained in the tube. At the end of the next two ten minute periods we drained out the continuous gastric secretion, as controls. We then placed on his tongue a very small amount of the sodium salt of saccharin, and at the end of the next two ten minute periods again collected the juice secreted. After 20 minutes with the sweet tasting saccharin in his mouth he began to eat his lunch, usually brought with him from his home, or selected at a nearby cafeteria. Ten and 20 minutes after starting to eat he collected the gastric juice. This gave us a 20-minute period which represented the continuous secretion,



a 20-minute period of appetite secretion with saccharin as a stimulous to the taste buds, and a 20-minute period of appetite secretion with the mastication and tasting of his food as the stimulus. The meal usually consisted of rolls, meat, milk and desert (pudding, pie or fruits). These experiments were con-

TABLE 4

Appetite Secretion of Gastric Juice in Mr. V. with saccharin in the mouth. Average of 14 experiments.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
Continuous secretion:				
10 minutes.....	2.5	.2158	.2550	3.25
10 minutes.....	3.2	.2173	.2552	3.4
Saccharin in mouth:				
10 minutes.....	3.5	.2290	.2723	3.81
10 minutes.....	4.4	.2616	.3091	3.8
Eating lunch:				
10 minutes.....	29.7	.3455	.3921	4.77
10 minutes.....	32.1	.3913	.4269	3.55

TABLE 5

Appetite Secretion of Gastric Juice in Mr. V. with sugar in the mouth. Average of 11 experiments.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
Continuous secretion:				
10 minutes.....	2.6	.1761	.2157	3.64
10 minutes.....	2.5	.1979	.2280	3.66
Sugar in mouth:				
10 minutes.....	6.9	.2434	.2881	4.2
10 minutes.....	7.1	.2862	.3237	4.14
Eating lunch:				
10 minutes.....	25.7	.3461	.3930	4.9
10 minutes.....	25.6	.3831	.4275	3.75

TABLE 6

Influence of Previous Ingestion of Saccharin and Cane Sugar on the Appetite Gastric Juice of Man.

	No. of experi- ment	Secretion rate aver. cc. per min.	Acidity of					
			Free			Total		
			Low	High	Aver.	Low	High	Aver.
Saccharin in Stomach..	15	3.3	0.40	0.50	0.44	0.47	0.56	0.51
Sugar in Stomach..	15	3.4	0.35	0.43	0.40	0.41	0.50	0.46

trolled and compared with similar experiments in which Mr. V. placed in his mouth a piece of pure rock candy instead of saccharin during the second 20-minute period.

Each sample of juice was carefully measured and a portion titrated for free and total acidity. One cubic centimeter was titrated against N/40 NaOH using dimethyl-amino-azo-benzene and phenolphthalein as indicators for the free and total acidity respectively. The peptic activity was measured by making a digestion mixture of 1 cc. of gastric juice in 20 cc. N/10 HCl. Egg albumin in the Mett's tubes was coagulated in boiling water for ten minutes and the digestion time was 24 hours at 37°C. In this series, fourteen experiments with saccharin are compared with eleven experiments with sugar (rock candy) in the mouth.

Mr. V. always stated that the saccharine had a bitter metallic after-taste and that he did not enjoy it. The sugar, of course, had no such effect. A comparison of the amounts in the tables shows very little difference between the continuous secretions, but between the sugar and saccharin secretions there is a difference of nearly 50% for the 20-minute period. *Saccharin does cause an appetite secretion, but it is much less than the sugar appetite secretion under the same conditions.* The secretion during eating the lunch is greater after tasting saccharin than after tasting sugar. This might be due to greater enjoyment of the meal by contrast and the desire to rid the bitter taste from his mouth by food. The difference in acidity and pepsin between the two series are slight and unimportant.

### *III. The influence of saccharin in the stomach on the secretion appetite gastric juice in man.*

In these experiments on our fistula man, Mr. V., the sodium salt of saccharin in 200 cc. of water was introduced through the fistula into the empty stomach one hour before meals. In the control experiments cane sugar in 200 cc. water was put into the empty stomach one hour before meals. The meals varied in character from day to day. The food ingredients were left entirely to Mr. V.'s choice. The saccharin tests and the control sugar tests were run on alternate days, and this fact together with the number of experiments neutralizes the variable factor of food palatability.

The quantities of saccharin introduced into the stomach in each experiment varied from 0.1 gr. to 0.5 gr., the quantity of cane sugar from 0.5 gr. to 10 gr.



The results of our fifteen saccharin tests and fifteen cane sugar controls are summarized in Table VI.

It is clear that saccharin, acting either locally in the stomach or after absorption, tends to increase the acidity of the appetite gastric juice. This is probably of no pathological or therapeutic importance in the case of healthy persons. But this influence of saccharin would probably aggravate the symptoms of so-called hyperacidity of persons with gastric and duodenal ulcers, or certain types of neurosis with gastric complications.

*IV. The influence of saccharin in the stomach on the secretion of gastric juice in dogs.*

Healthy female dogs were selected and an accessory pouch was made from the fundus of the stomach after the method of Pavlov (13). After recovery from the operation the juice was collected during the experiment by means of a perforated rubber tube with a rubber flange which caught the juice running down the sides of the tube. The tube was inserted into the pouch and held in place during the experiment by means of a canvas apron.

Dog I was used from the 18th of June to the 21st of August and was in excellent condition all that time. The usual experimental procedure was to feed 200 grams of fresh raw meat and collect the juice hourly for six hours. Water was offered at the time of the feeding. The amounts of juice were measured and the free and total acidity were determined by titrating one cubic centimeter against N/40 NaOH with dimethyl-amino-azo-benzene and phenolphthalein as indicators. The peptic activity was measured by the digestion of coagulated egg white in Mett's tubes. The digestion mixture was made with 1 cc. of juice in N/10 HC1, two Mett's tubes, and digested for 24 hours at 37°C. With this dog 12 experiments were performed without saccharin and these are considered normal, or controls, since five of them were done on days between saccharin feeding periods. Two experiments in which 0.25 gram and three in which 0.5 gram were given are averaged together in Table VII. The saccharin, enclosed in gelatin capsules, was given with the meat. This prevented the dogs from acquiring a distaste for their food on account of the saccharin. Five experiments were performed in which one gram of saccharin in gelatin capsule with the meat and seven in which 2 to 2.5 grams of saccharin were given. Five experiments were performed in

which 10 cc. of a 5% solution of saccharin in sterile Ringer's solution were injected into the saphenous vein.

With Dog II six experiments were performed without saccharin, which are considered the controls. The meal consisted of 200 grams of fresh raw meat and water, if desired. Following these were two experiments in which 0.25 gram of the sodium salt of saccharin was given, and then three in which 0.5 gram was given in gelatin capsule with the meal.

With Dog IV four experiments without saccharin were performed which were considered normal, or controls. The meal consisted of 150 grams of boiled lean meat without water. Three experiments were performed when 1.5 grams saccharin were given by gelatin capsule with the meal.

At the end of the series, in order to check up on the daily variability, two experiments were performed daily on Dogs I and IV for three days. In the morning each dog was given 100 grams of boiled meat and 200 cc. of water by stomach tube. The dogs were placed in holders and the juice collected hourly for three hours when the dogs were released. Each sample was analyzed for acidity and peptic activity. After three hours they were again given 100 grams of meat and 200 cc. of water containing one gram of saccharin by stomach tube. The juice was collected for three hours and analyzed as before. The order of giving saccharin was reversed to eliminate the difference between morning and afternoon. Dr. Ivy, working with these two dogs, gave them on other days an identical meal (meat and water) in the morning and afternoon and found only slight variation. Dog IV gave 71 cc. and 72 cc. with two trials on the same day. Tables 7-17 show the results obtained.

The above data show in Dog I an increase in amount and a rise in free and total acidity of the juice with each increase in dose of saccharin. Even the intravenous injection caused a rise in acidity and an increase in the amount of juice. There are daily variations but the averages are considered to represent the true effects of saccharin. The experiments tabulated in Table 17 largely eliminated the daily variation, and yet the same increase is shown. With Dog II the results show an increase in amount and a rise in free and total acidity. With Dog IV only those experiments were considered normal when the dog had a good appetite and ate its meat eagerly. The three saccharin experiments were performed after the controls, so that part of the large difference noted might

TABLE 7

DOG 1.—Control experiments in which no saccharin was given. Average of 12 experiments.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour.....	4.4	.17	.24	1.95
2nd hour.....	5.9	.31	.38	.94
3rd hour.....	8.2	.39	.43	.82
4th hour.....	7.4	.39	.44	.82
5th hour.....	4.5	.36	.42	.95
6th hour.....	4.6	.35	.42	.96
Total average.....	39.1	.33	.39	1.07

TABLE 8

DOG 1. Average of two experiments using 0.25 gram Na-Saccharin and three experiments using 0.5 gram Na-Saccharin in gelatin capsule with meal. Average of five experiments.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour.....	4.5	.18	.23	2.20
2nd hour.....	8.4	.37	.42	1.03
3rd hour.....	9.1	.40	.45	1.10
4th hour.....	8.5	.39	.45	1.05
5th hour.....	7.6	.42	.46	1.25
6th hour.....	5.4	.38	.44	1.18
Total average.....	43.5	.36	.41	1.30

TABLE 9

DOG 1.—Five experiments using one gram of Na-Saccharin fed in gelatin capsules with meal. Average of five experiments.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour.....	8.8	.32	.39	.99
2nd hour.....	10.7	.40	.45	.50
3rd hour.....	10.0	.38	.45	.58
4th hour.....	8.3	.39	.45	.71
5th hour.....	10.2	.40	.46	.92
6th hour.....	8.0	.35	.41	.9
Total average.....	50.2	.37	.43	.76

TABLE 10

DOG 1.—Seven experiments using 2 to 2.5 grams of Na-Saccharin in gelatin capsules with 200 grams of meat. Average of seven experiments.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour . . . . .	6.3	.27	.32	.75
2nd hour . . . . .	8.9	.39	.43	.48
3rd hour . . . . .	11.2	.39	.44	.45
4th hour . . . . .	8.3	.41	.45	.39
5th hour . . . . .	7.6	.42	.46	.50
6th hour . . . . .	8.0	.41	.45	.80
Total average . . . . .	53.2	.38	.43	.56

TABLE 11

DOG 1.—Average of five experiments with intravenous injection of 10 cc. of a 5% solution of Na-Saccharin in Ringer's solution.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour . . . . .	3.4	.26	.31	.101
2nd hour . . . . .	5.6	.29	.39	.8
3rd hour . . . . .	8.8	.40	.44	.20
4th hour . . . . .	8.5	.41	.46	.42
5th hour . . . . .	7.8	.38	.44	.35
6th hour . . . . .	5.2	.36	.44	.56
Total average . . . . .	41.1	.35	.41	.50

TABLE 12

DOG 1.—Control experiments without saccharin. 200 grams of fresh raw meat. Average of six experiments.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour . . . . .	5.5	.04	.15	3.67
2nd hour . . . . .	5.3	.20	.27	1.88
3rd hour . . . . .	1.11	.21	.27	2.07
4th hour . . . . .	5.1	.16	.24	2.1
5th hour . . . . .	4.4	.13	.22	1.77
6th hour . . . . .	4.9	.15	.23	1.6
Total average . . . . .	31.3	.15	.23	2.08

TABLE 13

DOG II.—Average of two experiments with 0.25 gram Na-Saccharin and three experiments with 0.5 gram Na-Saccharin, given in gelatin capsule with meal.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour.....	5.8	.12	.22	1.8
2nd hour.....	7.9	.24	.32	1.2
3rd hour.....	7.7	.22	.29	1.57
4th hour.....	4.4	.20	.27	1.55
5th hour.....	4.7	.15	.21	1.6
6th hour.....	3.8	.16	.22	1.55
Total average.....	35.6	.18	.25	1.54

TABLE 14

DOG IV.—Control experiments without saccharin. 150 grams of boiled meat with no water. Average of four experiments.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour.....	9.3	.30	.33	1.67
2nd hour.....	11.1	.38	.44	1.40
3rd hour.....	9.4	.36	.42	1.30
4th hour.....	9.1	.33	.40	1.19
5th hour.....	7.6	.32	.39	1.64
6th hour.....	2.3	.16	.33	1.52
Total average.....	49.0	.31	.39	1.45

TABLE 15

DOG IV.—Average of three experiments using 1.5 grams Na-Saccharin in gelatin capsule with meal.

	Amount cc.	% Free Acidity	% Total Acidity	mm. Digestion
1st hour.....	23.0	.40	.43	1.55
2nd hour.....	18.8	.44	.48	1.23
3rd hour.....	16.8	.44	.48	1.1
4th hour.....	14.2	.40	.45	1.3
5th hour.....	7.6	.38	.42	1.5
6th hour.....	5.5	.29	.35	1.6
Total average.....	86.6	.39	.43	1.4



TABLE 16

DOG I.—Average of six experiments, to check up the variability in the condition of the dogs. Meal consisted of 100 grams of boiled meat with 200 cc. of water by stomach tube. Time, three hours. After interval of three hours, repeated with addition of one gram Na-Saccharin to water. Order reversed on third day.

	Control—without Saccharin				With Saccharin			
	Amount cc.	% Free Acidity	% Total Acidity	mm. digestion	Amount cc.	% Free Acidity	% Total Acidity	mm. digestion
1st hour	6.6	.33	.37	0.8	9.4	.37	.41	.4
2nd hour	8.0	.37	.41	0.6	6.4	.34	.41	.4
3rd hour	3.4	.36	.41	0.5	6.4	.32	.38	.4
	18.1	.35	.40	6.3	21.3	.34	.40	.4

TABLE 17

DOG IV.—Average of six experiments to check up the variability in the condition of the dogs. Meal consisted of 100 grams of boiled meat with 200 cc. of water by stomach tube. Time, three hours. After interval of three hours, repeated with addition of one gram Na-Saccharin to water. Order reversed on second day.

	Control—Without Saccharin				With Saccharin			mm. Digestion
	Amount cc.	% Free Acidity	% Total Acidity	mm. digestion	Amount cc.	% Free Acidity	% Total Acidity	
1st hour	21.4	.33	.36	1.6	31.9	.42	.45	8
2nd hour	20.2	.40	.43	1.15	32.9	.45	.48	.7
3rd hour	11.9	.37	.40	1.1	16.1	.41	.45	.8
	53.6	.36	.40	1.2	81.0	.43	.46	.8

be due to the better condition of the animal. However, the experiments tabulated in Table 17 check this up and show that the increased secretion and acidity must be ascribed to the saccharin ingested.

The decrease in peptic activity is constant with the ingestion of saccharin throughout all these experiments with the exception of those tabulated in Table 7, for Dog I.

The results obtained with the peptic digestion experiments *in vitro* are in accordance with the results of the majority of previous investigators. The sodium salt of saccharin when added to the acid gastric juice is, in part, converted into the acid saccharin. But since the acidity of the digestion mixture with the low per cent. of saccharin is practically the same as the controls the inhibition is not due to the decreased acidity. We are inclined to believe with Salkowski that the inhibition is due to a specific drug action on pepsin.



The experiments on appetite gastric secretion on Mr. V. were carefully controlled. We therefore feel that these results represent the normal psychical effects on man. No reference in the literature was found on this phase of saccharin action.

The results on dogs largely eliminate the psychic factor except for the fact that saccharin in the blood is excreted in the saliva. It might be that the sapid taste in the mouth of the animals stimulates the gastric glands to greater activity, though in man this has the opposite effect, and it is doubtful if this is the true explanation. The rise in acidity is slight compared with the total quantity of acid present, and it is doubtful if this would have any pathological significance. The decrease in peptic activity suggests that saccharin in the blood circulating about the gastric glands might interfere with the zymogen formation, as no saccharin could be detected by taste in carefully neutralized juice when large doses of saccharin were given. The amounts of saccharin given to these animals, of course, is far in excess of any amount that would be used for ordinary sweetening purposes in food and drinks of man. Where saccharin is used as a substitute for sugar in preserves, syrups and candies, etc., individuals, especially children, might ingest corresponding quantities.

*V. The influence of saccharin on intestinal absorption.*

All of the experiments on this phase were made on large dogs under light ether anesthesia. The dogs were given water but no food for two days before the experiments, to insure an empty and otherwise uniform condition of the small intestines.

The small intestine was tied off in loops each twelve inches long. The fluids to be tested were introduced, at body temperature, into the loops with a hypodermic syringe and removed through a small orifice in the lower end of each loop. The absorption period in nearly all the experiments was fifteen minutes.

Special care was taken to subject the intestine to the least possible exposure, handling and temperature change.

In large dogs the small intestine yields ten 12 inch loops, as prepared by us. Our object in making many and rather short loops was to be able to obtain all the necessary controls on each animal, as well as control on the different regions of the intestines. The processes of secretion and absorption are not uniform for the entire length of the small gut. And as these processes may vary to a certain extent from day to day with the individual animal, it

seemed to us that Dog II cannot be used as control in absorption in the absence of saccharin for the results on Dog I with saccharin.

Our observations are subject to the strictures that may be made against all short or crucial experiments under anesthesia. If saccharin, taken in food or drinks, has any influence on intestinal absorption, it is a question of small quantities of the substance acting for months and years. But observations thus conducted would be complicated by the influence of saccharin on the taste, and on gastric digestion and secretion. We do not know any factor in the analysis of the blood, the urine, the feces or general metabolism that would give valid evidence of saccharin influence on intestinal absorption alone, unless this influence was very marked.

Our main aim in these experiments was to determine whether saccharin acts on the processes of absorption in any other way than as an indifferent crystalloid, that is, by osmosis.

The results on the seven dogs used in this series are given in Table 18. These data show clearly that *saccharin delays intestinal absorption of water by some other mechanism than the osmotic factor*.

In many of the experiments quantitative determinations were made of the sugar in the fluid recovered from the intestinal loops. These determinations showed more sugar when saccharin was part of the mixture, but it is not clear that this means delayed absorption of sugar. It may mean delayed destruction of sugar by the intestinal bacteria.

These actions of saccharin on gastric secretion and digestion, and on intestinal absorption seem to explain the gastro-intestinal disturbances described by Herter on ingestion of large quantities of saccharin by man.

#### VI. *The influence of saccharin on the kidneys.*

Folin fed saccharin to seven young men for a period of about five months varying from 0.15 to 0.75 grams per day. No material difference was noted in the metabolism. But no observations were made on men with impaired kidney function.

Herter fed saccharin to three men in amounts from 0.3 to 1.5 grams daily. With smaller amounts no disturbance of digestion or metabolism was observed. Larger doses—over 3 grams per

TABLE 18

Influence of Saccharin on the intestinal absorption of water.

Solution		Quantity Injected cc.	Quantity Recovered cc.
Dog I (7 kilos)	Water	15	0
	1% saccharin	15	6.5
	1% dextrose	15	0
	10% saccharin	15	34.0
Dog II (8 kilos)	1% cane sugar	60	7.5
	1% cane sugar in 0.5% saccharin	60	25.5
	1% dextrose	60	14.0
	1% dextrose in 0.5% saccharin	60	29.4
	1½% cane sugar	60	13.5
	1½% dextrose	60	16.0
Dog III (18 kilos)	1% dextrose	75	3.6
	1% cane sugar	75	3.0
	1% dextrose in 0.2% saccharin	75	10.5
	1% cane sugar in 0.2% saccharin	75	8.5
	1½% cane sugar	75	6.5
	1½% dextrose	75	5.0
Dog IV (12 kilos)	1% cane sugar	60	22.3
	1.2% cane sugar	60	20.2
	1.5% cane sugar	60	24.3
	1% dextrose	60	25.0
	1.2% dextrose	60	28.0
	1.5% dextrose	60	33.0
	1% cane sugar in 0.2% saccharin	60	29.5
	1% cane sugar in 0.5% saccharin	60	37.0
	1% dextrose in 0.2% saccharin	60	35.0
	1% dextrose in 0.5% saccharin	60	40.3
Dog V (10 kilos)	1% cane sugar	60	14.0
	1.2% cane sugar	60	14.0
	1.5% cane sugar	60	15.0
	1.2% dextrose	60	16.5
	1.5% dextrose	60	18.3
	1% cane sugar in 0.2% saccharin	60	25.0
	1% cane sugar in 0.5% saccharin	60	32.0
	1% dextrose in 0.2% saccharin	60	20.0
Dog VI (11 kilos)	1.1% cane sugar	60	1.5
	1.1% dextrose	60	3.0
	1% cane sugar in 0.1% saccharin	60	3.7
	1% dextrose in 0.2% saccharin	60	9.5
	1% dextrose in 0.2% saccharin	60	10.2
Dog VII (14 kilos)	Water	75	25.0
	0.025% dextrose	75	30.0
	0.025% saccharin	75	40.0
	0.050% dextrose	75	31.0
	0.050% saccharin	75	45.2
	0.100% saccharin	75	47.0
	0.200% dextrose	75	35.0
	0.200% saccharin	75	50.0

day—caused disturbances of digestion. The urine showed no significant change in nitrogen and sulphur metabolism.

The conclusions of Folin and Herter apply to healthy adults only. If saccharin is to be used as a substitute for sugar in foods and drinks, it will be digested by people of all ages and degrees of physiological impairments. The normal individual has at least twice the amount of kidney tissue actually required for adequate urine elimination. Hence the kidney tissues must suffer at least 50% impairment before that impairment can be demonstrated by physiological or clinical tests. It would therefore seem logical that the possible injurious action of saccharin on kidney activity should be investigated, on persons and experimental animals in whom the kidney "factors of safety" have been reduced by operation or disease.

#### *Experimental Procedure.*

Four healthy adult female dogs weighing from 8 to 10 kmg. were used in these experiments. By operative procedure the urethral orifices of the dogs were exposed so that they might be more easily catheterized. In Dog II the left kidney was removed. In Dog I about one-half of the left and at a subsequent operation the whole of the right kidney was removed. Dogs III and IV were left with both kidneys intact.

The dogs were kept in metabolism cages in which the urine was collected free from contamination, and every morning each dog was catheterized to secure accurately the 25 hour specimen of urine.

The dogs were given a standard diet consisting of round steak, 150 grams; beef suet, 100 grams; whole milk, 150 cc.; water, 200 cc. Some milk was mixed with the water so that the dogs might take the whole amount each day.

The saccharin was put in gelatin capsules and the capsules inserted in small cubes of meat. In this way the dogs took any amount without tasting it.

The pure sodium salt of saccharin was fed to the dogs in the following amounts:

Dog I (half kidney)

June 3-30—5 gr. per day.

July 1-5—10 gr. per day.

July 6-8—15 gr. per day.

July 9-15—20 gr. per day.

## Dog II (one kidney)

June 3-July 4—5 gr. per day.

July 5-15—10 gr. per day.

## Dog III (normal kidneys)

June 20-30—5 gr. per day.

July 1-4—10 gr. per day.

July 5-15—15 gr. per day.

## Dog IV (normal kidneys)

June 20-July 15—5 gr. per day.

Before the feeding of saccharin was begun, tests were made for the functional activity of the kidneys.

TABLE 19  
PHENOLSULPHONPHTHALEIN TEST

Dog I—(one-half kidney)

	Date	First Appearance	First Hour Amt. Excreted	Second Hour Amt. Excreted
Control.....	5-26	13 min.	41.66%	19.3%
Period.....	5-29	8 min.	44.8%	15.6%
.....	6-3	7 min.	45.63%	11.36%
Saccharin.....	6-10	7½ min.	42.6%	11.48%
.....	6-20	8 min.	43.5%	14.6%
Period.....	6-28	7 min.	42.0%	13.0%
.....				

TABLE 20  
PHENOLPHTHALEIN TESTS

Dog II—(one kidney)

Date	First Appearance	First Hour Amt. Excreted	Second Hour Amt. Excreted	
5-7	10 min.	50.7%	13.3%	Control Period
5-15	9 min.	45.3%	16.2%	
6-3	11 min.	46.0%	18.3%	Saccharin Period
6-10	9 min.	48.2%	15.6%	
6-28	8 min.	45.6%	14.9%	
7-6	9 min.	41.0%	17.4%	
7-21	11 min.	28.5%	26.0%	10 gr. saccharin injected
8-7	9 min.	30.0%	11.6%	10 gr. dextrose injected
8-9	9 min.	32.0%	31.6%	5 gr. saccharin injected



TABLE 21  
PHENOLPHTHALEIN TESTS  
Dog III—(normal kidneys)

Date	First Appearance	First Hour Amt. Excreted	Second Hour Amt. Excreted	
6-9	10 min.	35%	10%	Control Period
6-22	11 min.	36%	18.5%	
6-15	9 min.	39%	17.6%	
6-28	10 min.	40%	15.0%	Saccharin Period
7-6	12 min.	42.7%	21.6%	
7-21	10 min.	45.7%	15.6%	
8-7	9.5 min.	34.1%	21.1%	25 gr. saccharin injected
8-9	9.5 min.	50.0%	12.3%	½ gr. saccharin injected

TABLE 22  
PHENOLPHTHALEIN TESTS  
Dog IV—(normal kidneys)

Date	First Appearance	First Hour Amt. Excreted	Second Hour Amt. Excreted	
5-28	10 min.	48.6%	17.50%	Control Period
6-5	9 min.	46.5%	15.37%	
6-10	8.5 min.	47.3%	18.5%	
6-28	9 min.	46.5%	19.0%	Saccharin Period
7-6	6.5 min.	45.4%	13.3%	
7-21	8.5 min.	45.7%	16.4%	
8-7	8 min.	22.16%	29.41%	5 gr. saccharin injected
8-9	10.5 min.	50%	18%	1 gr. saccharin injected

### *Comments on the Results*

In none of the four dogs was there any evidence that the feeding of saccharin in amounts varying from 5 grams to 20 grams per dog for the periods indicated exerted a deleterious effect on the kidneys. The specific gravity of the urine was increased during the saccharin period due to the fact that saccharin is eliminated largely (75-90%) in the urine. At no time even with the largest doses and in the nephrectomized dogs was there any evidence of nephritis, as shown by urinary findings. The phenol-sulphonephthalein elimination was practically unchanged. But as even the half kidney dog (I) showed a kidney capacity equal



to that of a dog with two intact kidneys, *it is therefore evident that slight kidney impairment would not have been shown by these tests.*

Intravenous injections of the sodium salt of saccharin shortly before making the functional kidney test reveals a kidney action of the saccharin that may be of significance in cases of the use of saccharin by persons suffering from nephritis.

Ten grams of saccharin in 25 cc. of water were injected into the saphenous vein of the one kidney dog, and phenolsulphonethalein test done immediately, the amount of thalein excreted the first hour was reduced about one-half below normal, 28.5% being obtained the first hour and 26% the second hour. The intravenous injection of 10 grams dextrose does not interfere with the elimination of thalein. With 5 grams saccharin injected there was 32% eliminated the first hour and 31.6% the second hour, that is, a distinct diminution of the excretion the first hour. In the dog with two normal kidneys 25 grams saccharin injected intravenously, there was some interference with the thalein elimination during the first hour. One-half gram of saccharin appeared to have no effect. Similar results were obtained in Dog IV with two normal kidneys.

Our results show clearly that relatively large quantities of saccharin in the blood stream have a marked but temporary depressor action on the normal kidneys. Since similar or even much greater quantities of dextrose in the blood do not have this kidney action, *it would seem that the saccharin action is not through its osmotic factor, although osmosis probably plays a part.* The parallel experiments with dextrose are not entirely satisfactory as controls, because of the fact that the sugar molecules, besides being eliminated by the kidneys, are also oxidized or stored as glycogen in the tissues. It is, therefore, likely that the osmotic factor of dextrose in the blood is equalized more rapidly than that due to similar quantities of saccharin.

Since the influence of saccharin on cells in general (hemolysis, intestinal absorption) appears to be in the direction of decreased permeability, it is probable that this kidney action of saccharin is in part due to this factor.

It is not necessary to point out that in the case of persons suffering from nephritis, this influence of saccharin on kidney function, namely, decreased elimination through osmotic action and decreased permeability, might be significant. And while it is true

that large quantities of saccharin are necessary to bring out these deleterious processes in healthy dogs, smaller quantities of the substance might show the same effect in proportion to the severity of the disease. This question can be settled by actual observations on nephritic patients.

The objection that our ordinary articles of diet, such as proteins and salts, may act unfavorably in nephritis is beside the mark. Proteins and salts are necessary constituents of the diet, and in nephritis it is a question of balancing the nutrition requirements with the kidney capacity, so that the minimum detriment to the patient is produced. Saccharin is not a food, it yields no energy, its elimination means probably extra work on the part of the kidneys. *Hence, saccharin should not be permitted in the food and drinks of nephritic individuals, unless it can be shown that the quantity of the substance likely to be taken in this manner does not aggravate any stage or type of nephritis.*

#### VII. *The Action of Saccharin on Cells and Tissues.*

The marked action of even minute quantities of saccharin on the organs of taste, and the distinct antipeptic action of somewhat larger quantities of the substance appear to be the only physiological actions of saccharin clearly established by previous investigators. The fact that healthy individuals can ingest considerable quantities of the substance over long periods without evident harmful effect and the further fact that it is eliminated, quantitatively and unchanged, in the urine is generally taken to mean that saccharin has no action on the body cells in general, except possibly through the factor of osmotic pressure.

The following experiments on hemolysis were carried out with the view of determining whether this view is correct.

A 1 per cent. solution of the chemically pure sodium salt of saccharin lowers the freezing point  $0.19^{\circ}$  C. Samples of commercial saccharin showed usually a slightly lower osmotic pressure ( $-0.17^{\circ}$  C. -  $-0.18^{\circ}$  C.). A solution of chemically pure dextrose was made up to a strength that showed a lowering of the freezing point equal to that of 1 per cent. saccharin. In all our hemolysis tests the equimolecular dextrose solution was used as a control on the saccharin solution, on the assumption that the action of the dextrose on the erythrocytes is essentially one of osmosis.

Our results may be shown by the following typical experi-

ments, using in every case dog's defibrinated blood or washed erythrocytes (dog) suspended in Ringer's solution.

2 cc. 1.4% dextrose 1/10 cc. dog's corpuscles	}	Complete hemolysis in 9 minutes.
2 cc. 1% saccharin 1/10 cc. dog's corpuscles	}	Complete hemolysis in 15 minutes
2 cc. 1.4% dextrose 1 cc. 1% NaCl. 1/10 cc. dog's corpuscles	}	Marked hemolysis in 30 minutes.
2 cc. 1% saccharin 1 cc. 1% NaCl 1 10 cc. dogs corpusclss	}	No hemolysis in 30 minutes.
1 cc. 1.4% dextrose 2 cc. 1% NaCl. 1/10 cc. corpuscles 1 drop 3% saponin	}	Complete hemolysis in 25 minutes.
1 cc. 1% saccharin 2 cc. 1% NaCl. 1/10 cc. corpuscles 1 drop 3% saponin	}	Complete hemolysis in 45 minutes.
1 cc. 1.4% dextrose 1/10 cc. corpuscles 1 cc. bile salt solution	}	Complete hemolysis in 5 minutes.
1 cc. 1% saccharin 1/10 cc. corpuscles 1 cc. bile salt solution	}	Complete hemolysis in 15 minutes.
1 cc. 1.4% dextrose 1/10 cc. corpuscles 1/10 cc. ether	}	Complete hemolysis in 10 minutes.
1 cc. 1% saccharin 1/10 cc. corpuscles 1/10 cc. ether	}	Complete hemolysis in 30 minutes.

On the assumption that the influence of the dextrose molecule on the processes of hemolysis is essentially osmotic the above experiments show that *saccharin acts in other ways than by osmosis on the erythrocytes*. The apparent protection against hemolysis by saccharin may be due to a reduced permeability of the corpuscles, but the exact nature of this action of saccharin remains unknown.

#### VIII. *The Distribution of Ingested Saccharin in the Body Fluids and in the Secretions and Excretions.*

It has been shown by previous investigators that saccharin taken by mouth is practically all eliminated, unchanged, in the urine. It would seem of some importance to know how readily saccharin in the blood passes into the tissues, the lymph, the

cerebro-spinal fluid and possibly the digestive secretions. The persistence of the saccharin tests in the mouth suggests a re-secretion of saccharin in the saliva, or a rather tenacious union of the saccharin molecule with the taste end organs.

*1. The Distribution of Saccharin in the Lymph and the Cerebro-Spinal Fluid.*

These tests were made on five dogs, under light ether anesthesia, with cannulae in the thoracic and cervical lymph trunks. The weight of the dogs varied from 8-12 K. The sodium salt of saccharin was injected intravenously in quantities of 0.1 gr. to 0.5 gr. The presence of saccharin in the lymph was determined by the characteristic saccharin taste.

Our results were practically identical in the five experiments. The introduction of as little as 0.1 gr. saccharin into the blood leads to the appearance of saccharin in the thoracic and the neck lymph within a few minutes. In three out of the five experiments the saccharin was also detected in the cerebro-spinal fluid.

It is therefore clear that saccharin passes readily from the blood into the other body fluids and the various tissues, so that any physiological action that saccharin may possess will be exerted on the body cells in general. The passage of the saccharin from the blood into the lymph and the tissues, and *vice versa*, is probably determined by the concentration of the substance in the blood, so that when taken in very minute quantities by mouth, and especially in case of retarded intestinal absorption, the concentration of the saccharin in the blood may at no time be great enough for passage into the tissue cells and the lymph.

*2. The Excretion of Saccharin by the Salivary and the Lachrymal Glands.*

1. Experiments on dogs. Three dogs under light ether anesthesia were provided with cannulae in the ducts of the salivary glands and a slow continuous flow of saliva induced by minimal quantities of pilocarpin. Saccharin was injected intravenously in quantities of 0.2 gr., 0.3 gr. and 0.5 gr., respectively. In each case enough of the saccharin passed into the saliva to give it the distinct and characteristic saccharin taste. In the dog receiving 0.5 gr. saccharin, the saccharin taste was also in evidence in the lachrymal secretion, the taste of saccharin being almost strong enough to mark the normal salty taste of the fluid.

2. Observations on man. A number of tests were made on ourselves and on Mr. V. in order to determine whether saccharin



taken by mouth may also be re-secreted into the saliva. It is probable that the appearance of saccharin in the saliva is a question of its concentration in the blood.

In the case of Mr. V. the saccharin introduced into the stomach through the gastric fistula, and since his esophagus is completely closed, any saccharin taste in the mouth must come via the saliva. In the case of ourselves the saccharin was swallowed in capsules or introduced by stomach tube. The stomach tube method is not very satisfactory, as frequently enough of the saccharin solution adheres to the lower end of the tube to give saccharin taste in the mouth on withdrawal of the tube. The outside of the capsules filled with saccharin must also be absolutely free from the substance.

Saccharin introduced into the empty stomach in quantities of 0.5 gr. or more together with 100-200 cc. water usually gives a saccharin taste in the mouth after a delay of 10-30 minutes. Saccharin in smaller quantities did not give this taste to the saliva, and with food in the stomach even 1.0 gr. or 1.5 gr. usually failed to give the mouth response. It is evidently a question of rapidity of intestinal absorption. The persistence of the saccharin taste in the mouth after partaking of foods or drinks adulterated with saccharin is probably not due to saccharin in the saliva.

### *3. The Excretion of Saccharin in the Milk.*

The experiments were made on milking goats. In the first series we used two goats four weeks (I) and six weeks (II) after becoming fresh. In the second series the two goats were used within seven and ten days after giving birth to the kids.

The experiments were carried out as follows: Immediately after milking in the morning the saccharin was given dissolved in water, on the theory that most of the water would pass right into the true stomach rather than into the rumen. The goats were then fed or put into the pasture. The saccharin tests were made on the sample of milk secured in the evening. The goats did not object to the saccharin sweetened water.

### *Results.*

#### GOAT I.

- Gr. 1.0 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IA)
- 0.5 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IB)
- 0.2 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IC)

#### GOAT II.

- Gr. 1.0 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IIA)
- 0.5 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IIB)
- 0.1 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IIC)

These samples of goats' milk were submitted to the Chicago Food and Drug Inspection Laboratory of the Bureau of Chemistry. Dr. G. W. Hoover, Chief of the Laboratory, wrote me under date of Oct. 1, 1917, that "saccharin was found in milk samples IA and IIA, but not in the other milk samples"; that is, both goats, on being given 1.0 gr. of saccharin in water showed saccharin in the milk drawn nine hours later. When smaller quantities of saccharin were fed, the saccharin either failed to be excreted by the mammary glands, or else it was present in too small quantities to be detected by the methods employed. Under date of Oct. 29, 1917, Dr. G. W. Hoover wrote: "It is believed now that we can detect saccharin, chemically and by taste, in quantities as small as one part per million."

Following this information, we proceeded with the experiments on goats III and IV.

#### GOAT III.

- Gr. 2.0 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IIIA)
- 1.0 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IIIB)
- 0.3 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IIIC)
- 0.2 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IIID)
- 0.1 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IIIE)

#### GOAT IV.

- Gr. 1.0 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IVA)
- 0.5 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IVB)
- 0.3 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IVC)
- 0.2 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IVD)
- 0.1 Saccharin in water at 8 A.M., milked at 5 P.M. (Sample IVE)

These samples were submitted to the Chicago Laboratory, Bureau of Chemistry, for analysis for saccharin. Under date of Nov. 26, 1917, Dr. A. E. Paul, Acting Chief of the Chicago Station, reports as follows:

Sample Marked	Saccharin (qualitative test)	
	Taste	Schmidt
IIIA	positive	positive
IIIB	doubtful	positive
IIIC	doubtful	positive
IIID	negative	negative
IIIE	negative	negative
IVA	positive	positive
IVB	positive	positive
IVC	doubtful	positive
IVD	negative	negative
IVE	negative	negative



"Saccharin is undoubtedly present in Samples IIIA, IIIB, IIIC. Judging from the strength of the qualitative test, the amount in IIIA is much larger than in IIIB, which in turn is larger than IIIC. I should judge the amount in the two latter to be about .05 to .1 mg. per 100 cc."

"Samples IIID and IIIE may contain traces, but less than one part per million."

"Samples IVA, IVB and IVC contain saccharin. As in IIIA series IVA contains a much larger amount than IVB and IVC. The amount in IVB and IVC appears to be .05 to .1 mg. per 100 cc. Samples IVD and IVE do not contain saccharin."

These experiments leave no doubt about saccharin, taken by mouth in sufficient quantities, passes partly into the milk. The maximum quantities of saccharin that may be ingested by nursing mothers without passing into the mother's milk, must be determined by actual tests. It is probable that the absorption of saccharin is more rapid in man than in the ruminants, so that the same quantities of saccharin ingested will lead to a higher concentration of saccharin in the blood in women than in goats. The passage of saccharin into milk is probably a question of its concentration in the blood.

#### *Summary.*

1. The prevailing view that, except for its action on the organs of taste in the mouth, saccharin is an inert substance, having no action on organs and tissues, is not tenable. Saccharin acting in the mouth decreases appetite gastric secretion, acting in the stomach it increases gastric secretion, and decreases peptic digestion, acting in the small intestine it decreases absorption, acting on the erythrocytes it decreases hemolysis. These actions of saccharin cannot be explained by the osmotic factor.

2. Saccharin in the blood, in proportion to its concentration, passes into the lymph, cerebrospinal fluid, saliva, tears and mammary secretion.

#### *General Conclusion.*

The harmlessness of a substance like saccharin that may be used as a general substitute for sugar in foods and drinks cannot be established by short time feeding tests on healthy persons, since the healthy man has so large factors of safety that great organ deterioration must be produced before objective evidence

of physiological impairment is secured. In matters of public health and permissible food substitutes, scientists and statesmen should recognize that the weak as well as the strong eat common foods, and that feeding tests of 6-7 months' duration covers only a small part of the space of human life. If saccharin is permitted as a general substitute for sugar, saccharin will be ingested by old and young, in all stages of health, and for generations. There is at present no evidence that warrants the conclusion that saccharin even in very small quantities so taken is harmless. It would seem the part of wisdom to prohibit the use of saccharin in foods and drinks (except as ordered by the physician in diabetes) until such scientific evidence is secured.

## REFERENCES.

- Becht: *Jour. Pharm. Exp. Therap.*, 16, 1920, 155.  
Best: *Münch. Med. Woch.*, 64, 1917, 1231.  
Brauwardel, Peuchet and Argier: *Amer. Hyg. Pub.*, 1888, 300.  
Folin: *U. S. Dept. of Agriculture, Rep. No. 94*, Washington, 1911.  
Gaus: *Berl. Kl. Woch.*, 1889, 281.  
Heitler: *Wien. Med. Woch.*, 70, 1920, 1050, 1839.  
Herter: *U. S. Dept. of Agriculture, Rep. No. 94*, Washington, 1911.  
Jensen: *Arch. of Hyg.*, 10, 1890, 64.  
Nenki: Quoted by Herter.  
Paul: *Bull. Acad. Med.*, 20, 1888, 32.  
Petschek and Lerner: *Centrabl. f. d. ges. Ther.*, 1889, 321.  
Plugge: *Schmidt's Jahrb.*, 221, 1889, 140.  
Roger and Garnier: *Arch. Med. Exp.*, 19, 1907, 497.  
Salkowski: *Virchow's Arch.*, 105, 1886, 46.  
Schmitt: *Compt. Rend. Soc. Biol.*, 53, 1901, 373.



## DIET TREATMENT OF DIABETES INSIPIDUS

FREDERICK M. ALLEN, M.D.,

AND

JAMES W. SHERRILL, M.D.

*The Physiatrie Institute, Morristown, New Jersey.*

Diabetes insipidus is still covered by the symptomatic definition originally given to it by Johann Peter Frank in 1794, "a prolonged excessive secretion of non-saccharine urine not due to kidney disease." The fundamental disturbance must be acknowledged as still unknown, and there is uncertainty even whether it is a single entity or whether several disorders are included under the one head. The theory of the hypophyseal origin has become the leading one, on the basis of well-known experimental work extending from Schafer to Cushing<sup>20</sup>, but on the other hand a survey of the literature shows a persistent and apparently growing contention that mid-brain lesions are the essential factor and that the hypophysis has absolutely no specific relation to the condition<sup>2, 3, 21</sup>. The pathological physiology is still more doubtful. Under the influence of Koranyi's physical views of renal function, Tallqvist<sup>35</sup>, Meyer<sup>22, 23</sup> and others sought an explanation in an inability of the kidneys to concentrate urine, which for some years was the dominant theory. More recently, however, opponents have shown (1) that tests under some conditions demonstrate a retention of some degree of concentrating power<sup>3, 5, 10, 11, 12, 13, 14, 15, 19, 20, 21, 27, 32</sup>; (2) that a fairly high degree of concentration is obtained with intercurrent disturbances such as fever or narcosis<sup>15, 18, 21</sup>; (3) that phenomena of retention or uremia<sup>21</sup> are absent or trivial in comparison with the dangers of this kind which accompany the true hyposthenuria of Bright's disease<sup>2, 3, 17, 27, 29</sup>; (4) that the molecular concentration of the urine may be lower than that of the blood plasma<sup>20, 24</sup>. Counter-arguments have been presented to some extent against the first three objections, but the fourth seems to be decisive. With complete isosthenuria, elimination of solids demands enough water to make the osmotic tension of the urine equal to that of the blood; but when thirst

compels the drinking of water considerably in excess of this amount, the theory that the water is required merely as the vehicle for the excretion of solids under physical laws must fall. Some authors now believe that more than one factor may be primary in the same or different cases<sup>3, 38, 39, 40</sup>. Likewise, though thirst has been found to precede polyuria in some cases<sup>3, 41</sup>, the old question of primary polydipsia or polyuria remains unsettled<sup>9, 12, 34</sup> to such an extent that some writers have considered the distinction impossible or assume different conditions in different cases<sup>3, 21, 38</sup>. Granting the existence of a primary polyuria, it seems not explainable by deficient absorption in the renal tubules, and therefore is interpreted by some as a true tubular hydrorrhea<sup>18, 19, 21, 27</sup>. Others have suggested an abnormal sensitiveness of the kidneys to stimulation by excretory substances<sup>13, 14, 15</sup>. There is a further question whether the epithelium or the vascular apparatus of the kidneys is concerned<sup>27</sup>. Opponents of the hypophyseal theory consider it improbable that the kidney function is so largely controlled by a distant gland<sup>27</sup>, and regard the action of pituitary extract as a non-specific drug effect<sup>21, 40</sup>. Upholders of this theory are undecided as to the mechanism of the supposed hypophyseal regulation<sup>20, 26</sup>. In addition to direct renal influences and direct nervous influences, attention has been given recently to possible abnormalities of the general water balance of the body. Some authors believe in a primary alteration of the equilibrium between blood and tissues<sup>21, 24, 38, 39, 40</sup>, but the best evidence seems to oppose this view and also to exclude either hydremia or a lowered water threshold of the kidneys<sup>27</sup> as the essential feature<sup>2, 3, 5, 8, 19, 41</sup>.

The therapy is in an equally unsatisfactory state. Temporary or permanent cures of diabetes insipidus by lumbar puncture have been reported in a few cases<sup>8, 36</sup>. The rationale of this treatment is not clear, but such cases evidently differ in some way from the great majority which cannot thus be relieved<sup>6, 41</sup>. The control of thirst and polyuria by subcutaneous or intranasal<sup>6</sup> applications of pituitary extract seems to have been chiefly of theoretical interest as used for patients in hospital<sup>3, 4, 20, 21, 29, 33, 41</sup>. This method seems not to have been considered generally feasible for long continued practical treatment, though some have recommended a single dose of pituitrin in the evening for relief of nocturnal polyuria. Rees and Olmstead<sup>30</sup> have reported marked benefit in



one case from the giving of 2 grains of desiccated posterior lobe of pituitary in salol coated capsules t. i. d. a. c., though the feeding of extract without the salol covering was ineffectual, supposedly because of destruction in the stomach. It is conceivable that the success reported by Motzfeldt<sup>26</sup> with the feeding of as much as seven fresh posterior lobes every evening may be explainable by the assumption that a portion of this material escaped gastric digestion. Blumgart<sup>6</sup> obtained negative results with salol coated tablets. On the whole, pituitary treatment has not yet found successful application for the prolonged control of diabetes insipidus.

Variations in the intake of water, salts and nitrogen have constituted one of the favorite experimental methods, and have also been applied therapeutically. It is generally recognized that cases which are controlled by mere withdrawal<sup>10</sup> of water are of hysterical nature, and that the intolerable thirst and serious symptoms resulting from this measure furnish one of the best diagnostic signs of true diabetes insipidus. The restrictions of sodium chloride and of protein date back to Tallqvist, and were thus based upon the same principles which have proved their value in kidney disease, but the results in diabetes insipidus have on the whole not fulfilled expectations, as shown by the following partial review.

The dietary restrictions of Tallqvist<sup>28</sup> and Meyer<sup>22</sup> were not very rigid, but they reported clinical benefits.

Engel<sup>10</sup> furnished examples of diets which reduced the urinary nitrogen from 12.1 to 8 gm. and the sodium chloride from 13 to 2.5 gm., with an attendant reduction of urinary volume from 6,200 to 3,500 cc.

Minkowski<sup>25</sup>, though accepting Tallqvist's theory, described variable results with diet. In one instance limitation of salt and protein reduced the urine from 12 or 14 to 3 or 4 liters. In another case, however, a boy's diet was restricted so that his urinary chloride fell to 0.01 per cent, while the thirst and polyuria were scarcely affected.

Forschbach and Weber<sup>15</sup> observed alterations of the volume and gravity of the urine with diet changes, chiefly on an experimental basis, but the sodium chloride of the urine seems not to have been reduced below 3.84 gm. on any day. Similar observations of others, having theoretical rather than therapeutic purposes, will be omitted.

Fitz<sup>12</sup> described considerable subjective and objective benefits from moderate dietary limitations.

Grote<sup>18</sup> stressed both the benefits and the limitations of dietary treatment. The limitations mentioned, however, are possibly explained by his



tables, which show that his diets furnished 7 gm. or more of nitrogen and 3 gm. or more of sodium chloride in the daily urine.

Bullowa<sup>7</sup> reported decrease of urine and rise of specific gravity not above 1,007 with salt restriction in a boy with diabetes insipidus, but definite data of diet and diuresis are not contained in the available abstract.

Bergé and Schulman<sup>4</sup> studied a case in which dietary changes were said to have little influence on the urinary secretion.

Kennaway and Mottram<sup>20</sup> tried restrictions of salt and protein in two cases. In the first one, the urine volume was reduced from about 8 to about 6 liters. "As the daily excretion of nitrogen now stood at only 4 gm., so that no further reduction could be expected, and that of chlorine at 2 gm., or about one-fifth the output of a person on ordinary diet, it is evident that no great success attended this form of treatment." In Case 2, the tables show that the urinary nitrogen remained above 9 gm. and the chlorine was not reduced below 4 or 5 gm. "The volume of urine fell from nearly 9 to 5 liters. The effect, however, was transient, and the volume of urine soon rose again to 7 liters." Following doses of sodium chloride, the volume of the urine was increased, its chloride concentration also increased, while the nitrogen content became more dilute, as found by most other investigators.

Bauer and Aschner<sup>3</sup> stated that dietary changes had no effect in the single case studied by them. Their second table shows reduction of the sodium chloride of the urine from 8.9 to 4.8 gm., and a range of urinary volume from 15 to 9 liters. It is not possible to decide whether the results of diet were really negative or whether their restrictions were not sufficiently rigid or prolonged.

Stenström<sup>22</sup> in one case reduced the sodium chloride of the urine as low as 1.5 to 3 gm. daily and the nitrogen as low as 4 gm. daily by dietary restrictions over a long period. He then found that addition of meat, to raise the nitrogen output to 12 gm., caused no increase of urine volume; but the freezing point, which had been -0.14 to -0.17, fell to -0.36°. He refers to the observations of authors, from Tallqvist onward, who found that the urine volume was increased when its nitrogen was increased by the giving of meat or urea; but he shows by the recorded figures and also by his own experiments that this result may be attributed to the increased elimination of chloride which is brought about by the extra nitrogen. When this chloride diuresis is prevented by a preliminary diet which exhausts the chloride reserves of the body, his findings with protein and those of Norgaard with 20 gm. urea show that the extra water diuresis is also lacking. Observations in two other cases were apparently confirmatory, though incomplete. He therefore concludes that the kidneys in diabetes insipidus are deficient in concentrating power only for sodium chloride. He considers that stringent limitations of salt and protein are almost impossible in prolonged practical treatment, but suggests that a sufficiently pleasing diet may be possible with moderate allowances of protein and avoidance only of salt.

Turning to textbook articles, it is found that Umber<sup>27</sup> follows Tallqvist and considers limitation of salt and protein the best general treatment. Fletcher's<sup>28</sup> position is similar but his allowances liberal. "A patient may be kept for months on a daily intake of 5 to 6 grams of salt without any injurious results. The protein should not be reduced below 50 grams daily. The essential feature in the diet is the moderate restriction of salt and protein." Palmer<sup>29</sup> remarks briefly that "Reduction of the salt and protein ingestion has served to diminish the severity of the symptoms in certain cases." Rowntree<sup>30</sup>, after discussing lumbar puncture, pituitrin and various drugs, says: "Dietary control plays a minor role. The ingestion of fluids may be gradually and judiciously cut down until further reduction fails to further decrease the urinary output. Beyond this it is unsafe to go, since undue reduction of fluids may prove disastrous. The thirst is best met by acidulated drinks in many instances. Similarly, control of salt and protein may be exercised."

Several considerations led us to try stringent dietary control. It is evident that the diets which have given incomplete or negative results in diabetes insipidus are of the same sort which have so commonly failed to relieve hypertension. We have demonstrated both the feasibility and the benefit of strict salt exclusion in most hypertension cases<sup>1</sup>, and similar observations on diabetes insipidus seemed to offer both practical and theoretical interest. If a series of authors are correct in contending that diabetes insipidus is not of hypophyseal origin, then pituitary extract is only a palliative drug and not a causal therapy. But if a lack of concentrating power of the kidneys is the immediate cause of, or an important factor in, the polyuria of this disorder, limitation of the materials for excretion is an evident causal treatment. If, according to a different theory, the kidneys are abnormally sensitive to small quantities of salt or nitrogen, it becomes all the more imperative to reduce these quantities to an actual minimum. If there is a disorder of intermediary metabolism, so that thirst is created by abnormal concentrations of sodium chloride either in the blood or in the tissues, these concentrations and the accompanying thirst should be more or less reduced by sufficient reduction of the salt supply. Veil divided diabetes insipidus into hyperchloremic and hypochloremic forms. These and all the earlier analyses of either whole blood or serum may be of doubtful value, but Rabinowitch found an elevated plasma chloride of 627 mg. per 100 cc. by Whitehorn's method as the only chemical abnormality in his case. In Bauer and Aschner's case the plasma chloride varied, but was often above 600 mg. Weir, Larson and Rowntree reported figures as high as 629 and 640 mg. Granting

that all the assumed abnormalities regarding salt or nitrogen are erroneous or secondary, and that diabetes insipidus may be a primary polydipsia from purely nervous cause, or a primary polyuria due strictly to excessive escape of water through the kidneys, some benefit may still be expected from dietotherapy on the principle that no organism, whether normal or abnormal, can be as thirsty without salt as with salt. If cases are found which are actually unaffected by dietary changes, as claimed by some authors, there may be interest in the distinction between these and the cases which are controllable by diet, and tests may perhaps indicate whether the refractory cases are merely more severe or organic (like the refractory cases of hypertension) or whether they constitute a separate type of disorder. On the other hand, there is the undecided question whether diabetes insipidus patients can endure such limitations of salt intake as can be borne by normal persons or nephritics.

#### CASE I.

The first of our four patients was a soldier treated on the diabetic service of U. S. A. General Hospital No. 9 in 1918. He appeared normal in all other respects, and gave no history or signs of syphilis or other known causes. No radiograms of the skull were taken. The case was moderate, with urine volume of 5 to 6 liters per day. The detailed records are not now available, but the urine was free from sugar or albumin, the glucose tolerance was normal, no deficiency of kidney function was shown by phenolsulphonephthalein or Ambard tests, and attempts to restrict the water drinking had failed. Salt restriction which reduced the urinary chloride to about 1 gm. per day gave complete symptomatic relief, by reducing the urine volume to about 2 liters per day, and no special limitation of protein was found necessary.

#### CASE II. (No. 879).

Male. Hebrew. Married. Age 43. Silk weaver. Admitted Oct. 10, 1921. Re-admitted June 5, 1922.

*Family History:* Mother living and well at 61. Father died of accident at 40. Five sisters and three brothers living and well. Patient married 18 years. Wife and four children living and well. There is no history of diabetes mellitus or diabetes insipidus in the family.

*Past History:* Measles, mumps and scarlet fever as a child. Has always been healthy, and has followed his occupation of silk weaver for the past 15 years. Has never suffered from headaches or dizziness. Fifteen years ago had lobar pneumonia. Two years ago suffered from tonsillitis and peritonsillar abscess, which was opened and drained. Denies

venereal diseases. Has never suffered from polyuria or nocturia. His diet has consisted mostly of meat, bread and vegetables. He has always used excessive quantities of salt.

*Present Illness:* The onset was in June of 1921. The first symptom was thirst, which was distressing. He noticed that it was necessary to drink water every 15 to 30 minutes. His weight decreased by 4 pounds during the first week. He was unable to sleep on account of dryness of the mouth and polyuria. He gradually grew weaker and within 4 weeks was unable to work. He consulted a physician, who suspected diabetes mellitus, but the blood and urine were found normal for sugar. He was told to restrict water, but after 48 hours thirst became intolerable. The diet was not altered. For one week he was given hypodermic injection of pituitrin, which relieved the polyuria appreciably. For the past 6 weeks he has received no medical care, and has taken an ordinary diet. The urine volumes have ranged from 6 to 10 quarts, with no tendency to improvement.

*Physical Examination:* The patient is an adult male of normal stature and appearance. He is fairly well nourished, but shows signs of loss of weight. He is seriously distressed by dryness of the mouth and fatigue. He shows evidences of loss of sleep. Eyes, ears and nose normal. Teeth in good condition. The tongue, palate and pharynx dry. Thyroid not palpable. Heart, lungs and abdomen normal. Extremities negative. Reflexes present and equal. Diagnosis: Diabetes insipidus.

*Laboratory Findings:* Urine: pale, colorless, neutral, Sp. Gr. 1005, very faint trace of albumin, no casts, negative for sugar, acetone and diacetic acid. Blood: urea 20 mg. per 100 cc., sugar 120 mg. per 100 cc., NaCl 590 mg. per 100 cc., Wassermann negative.

## DISCUSSION OF CASE II.

On admission, the patient was excreting over 7 liters of urine with 5.2 gm. NaCl in 24 hours. Limitation of drinking was prevented by thirst. The intake of sodium chloride was kept at 5 gm. on Oct. 12 and 13. With complete withdrawal of salt beginning Oct. 14, there was a prompt fall in the chloride output and also in the urinary volume, but in general the reduction of volume was not great and the result on this basis might be regarded as a therapeutic failure. The diet during this time was unweighed and was chosen by the patient entirely according to his own wishes except in regard to salt. The protein intake was judged only by the urinary nitrogen output, which was fairly constant between 10 and 12 gm. daily.

Beginning Oct. 21, the protein was restricted to 30 gm. daily, while carbohydrate and fat were still allowed ad libitum. Table 1 shows that though the chloride excretion remained unchanged, a well-marked reduction of urinary volume resulted. By Nov. 2 the excretion had fallen to 2400 cc. per day, without any compulsory limitation of drinking.





Keeping the protein at 30 gm. daily, from Nov. 3 to 8 the carbohydrate was limited to 30 gm., while the patient took as much fat as he could eat. Nov. 10 to 13, the fat was limited to 30 gm. daily, while carbohydrate was given in abundance. Extraneous circumstances prevented accurate urine records during this time, but the observations were sufficient to show that the influence of carbohydrate or fat was negative. The blood analyses likewise remained normal.

By Nov. 12 the urine volume had been reduced to 1450 cc. in 24 hours, with a content of 0.47 gm. NaCl. The patient was discharged the following day, entirely comfortable on his salt-free diet with 30 gm. protein and unlimited carbohydrate and fat.

TABLE 2. CASE II.

## TOLERANCE TEST.

Case No. 879 With 100 gm. of Glucose by Mouth.

	Plasma Sugar	Urine Sugar
Before Administration	130	0
½ Hour After	189	0
1 " "	171	0
2 " "	079	0
3 " "	085	0
4 " "	113	0

Table 2 shows that the glucose tolerance of this patient was normal, thus confirming the observations with the high carbohydrate diets in Table 1.

TABLE 3. CASE II.

Case No. 879. SALT TEST. November 11, 1921.

Hour Period	BLOOD		URINE			Fluid Intake cc.
	Plasma Sugar mg. per	Plasma NaCl 100 cc.	Vol. cc.	NaCl %	Total NaCl gm.	
1	-----	-----	-----	-----	-----	---
2	-----	-----	220	.004	.008	200
3	-----	568	225	.004	.008	200
4	107	583	270	.008	.021	200
5	-----	610	240	.006	.012	200
6	101	601	315	.020	.061	200
7	10 grams	salt by mouth.	-----	-----	-----	---
8	-----	-----	220	.029	.063	200
9	075	614	225	.024	.054	200
10	106	589	190	.024	.045	200
11	156	552	230	.024	.055	200
12	-----	-----	165	.020	.033	200

Table 3 shows the effects of a single dose of 10 gm. sodium chloride, after the patient had become thoroughly adjusted to a salt-free diet. There was neither polyuria nor any considerable excretion of the chloride



within the 5 hours of observation. The plasma chloride analyses gave no evidence of retention in the blood. These results agree with those in the literature which show bradyuria in diabetes insipidus, perhaps dependent upon a delayed absorption from the bowel.

TABLE 4. CASE II.

Case No. 879.

PROTEIN TEST.

November 9, 1921

Hour Period	BLOOD			URINE					Water Intake cc.
	Plasma Sugar mg.	Plasma NaCl per 100	Blood Urea cc.	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl gm.	Urea mg. per 100 cc.	
1	-----	-----	-----	-----	-----	-----	-----	-----	-----
2	-----	-----	-----	275	1005	.029	.079	222	200
3	-----	-----	-----	315	1007	.020	.062	144	200
4	-----	-----	-----	150	1007	.001	.003	156	200
5	093 50 gm.	526 protein.	12 No	260 salt.	1006	.007	.018	168	200
6	-----	-----	-----	255	1005	.001	.002	120	200
7	-----	-----	-----	220	1006	.001	.002	240	200
8	-----	-----	-----	215	1006	.001	.002	264	200
9	125	526	14	295	1006	.001	.003	468	200
10	-----	-----	-----	155	1007	.001	.001	504	200
11	122	526	11	165	1005	.003	.004	732	200
12	-----	-----	-----	145	1005	.001	.001	768	200
13	-----	540	36	205	1006	.003	.006	468	200
14	-----	-----	-----	155	1006	.003	.004	660	200
15	079	540	19	165	1006	.001	.001	540	200

Table 4 shows the results of a feeding of 50 gm. protein at a time when the patient had become adjusted to a salt-free diet and a low protein intake of 30 gm. per day. The urea output was increased, but the volume, sodium chloride and specific gravity of the urine remained unaffected. The fixed water intake of 200 cc. per hour abundantly satisfied thirst. If the experiment had been differently planned, allowing the patient to drink at will, it is possible that the extra protein might have created increased thirst and diuresis.

Table 5 shows the usual effect of pituitrin in reducing the volume and raising the concentration of the urine, even when the water intake is kept constant. Hyperglycemia was marked. The blood urea was scarcely affected, and the plasma chloride fell. The corpuscle volume was high, but the hemoglobin nevertheless was only 80 per cent. Both fell, indicating dilution of the blood by the retained water.

TABLE 5. CASE II.  
PITUITRIN TEST

Case No. 879.

June 25, 1922.

Time	BLOOD					URINE				Water Intake cc.
	Plasma Sugar mg.	Plasma NaCl per 100	Blood Urea cc.	R. C. V. %	Hgb. %	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl gm.	Albumin
6 to 7 A. M.	.....	.....	.....	.....	.....	87	1007	.029	.025	Ft. Trace
7 to 8	.....	.....	.....	.....	.....	110	1007	.012	.013	"
8 to 9	.....	.....	.....	.....	.....	351	1006	.012	.042	"
9 to 10	.....	.....	.....	.....	.....	275	1006	.016	.044	"
10 to 11	.....	.....	.....	.....	.....	319	1006	.019	.060	"
11 to 12	.....	.....	.....	.....	.....	240	1006	.082	.198	"
12 to 1	110	556	36	48.74	80	301	1006	.041	.123	"
1 to 2	.....	.....	.....	.....	.....	175	1008	.020	.035	"
2 P. M.	0.5 cc.	pituitrin	by hypodermic	.....	.....	.....	.....	.....	.....	.....
2 to 3	341	502	42	40.78	70	47	1020	.049	.023	"
3 to 4	150	536	36	45.45	75	70	1016	.078	.054	"
4 to 5	.....	.....	.....	.....	.....	55	1016	.051	.028	"
5 to 6	.....	.....	.....	.....	.....	75	1010	.037	.027	0
6 to 7	.....	.....	.....	.....	.....	65	1011	.029	.018	Ft. Trace
7 to 8	.....	.....	.....	.....	.....	80	1012	.020	.016	"
8 to 9	.....	.....	.....	.....	.....	137	1009	.020	.027	Trace
9 to 10	.....	.....	.....	.....	.....	120	1008	.024	.016	Ft. Trace
10 to 11	.....	.....	.....	.....	.....	95	1008	.016	.015	0

TABLE 6. CASE 11.

Second Admission

Case No. 879	DIET					URINE					BLOOD			Weight, lb.
	Protein gm.	Fat gm.	C. H. gm.	NaCl gm.	Total Cal.	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl gm.	T. N.	Plasma Sugar mg.	Plasma NaCl per 100	Blood Urea cc.	
Date 1922														
June 5	Unweighed													
6	90	144	260	0	2700	8000	1005	.051	4.08	13.88	112	610	.....	136
7	"	"	"	0	"	5400	1005	.047	2.53	14.37	.....	.....	.....	136
8	"	"	"	0	"	5000	1005	.041	2.05	12.40	.....	.....	.....	.....
9	0	188	250	0	"	3450	1006	.037	.127	9.51	120	570	28	135
10	"	"	"	0	"	2580	1005	.020	.51	7.74	.....	.....	.....	.....
11	"	172	286	0	"	2830	1005	.090	.82	6.19	.....	.....	.....	136
12	180	108	250	0	"	4900	1004	.012	.58	25.56	117	566	14	135
13	"	104	260	0	"	5270	1004	.020	1.05	27.30	.....	.....	.....	134
14	.....	.....	.....	.....	.....	3960	1006	.016	.63	16.59	.....	.....	.....	.....
15	90	144	"	0	"	3710	1007	.012	.44	11.27	.....	.....	.....	134
16	"	"	260	0	"	4020	1005	.022	.88	15.44	120	544	22	134
17	"	"	"	0	"	2948	1005	.....	.....	13.90	.....	.....	.....	134
18	"	"	"	0	"	3780	1005	.....	.....	14.50	.....	.....	.....	.....
19	"	"	"	0	"	3560	1006	.016	.56	14.47	.....	.....	.....	134
20	"	"	"	0	"	2565	1006	.029	.73	12.60	104	515	34	134
21	"	"	"	0	"	4190	1005	.029	1.21	15.33	.....	.....	.....	.....
22	"	"	"	0	"	2500	1008	.016	.40	11.40	.....	.....	.....	134
23	"	"	"	0	"	3360	1004	.016	.53	12.23	.....	.....	.....	134
24	"	"	"	0	"	3130	1007	.049	1.53	12.30	.....	.....	.....	.....
25	"	"	"	0	"	4012	1009	.117	1.63	14.40	110	556	36	134
26	"	"	"	0	"	2905	1007	.026	.75	13.33	.....	.....	.....	.....
27	"	"	"	0	"	3800	1004	.020	.70	14.45	.....	.....	.....	134
28	"	"	"	0	"	4120	1004	.026	1.07	.....	157	.....	.....	.....
29	"	"	"	0	"	.....	1005	.043	.....	.....	.....	.....	.....	134

After discharge, the patient was thoroughly comfortable and free from polyuria on his diet at home, until a change in his living conditions necessitated the breaking off of his accurate regime. When he took most of his meals in restaurants, notwithstanding his attempts to be careful, he suffered a return of thirst, polyuria and nocturia. He was therefore re-admitted June 5, 1922, in much the same condition as at the beginning. (Table 6). On a weighed diet without salt, the urine volume fell appreciably, but nevertheless remained uncomfortably high. The reason may be found in the protein intake of 90 gm. daily. June 9 to 11, all protein was omitted from the diet, while the same caloric intake was made up with carbohydrate and fat. The urine volume thus fell from 5000 to 2830 cc. On each of the following two days, June 12 and 13, 180 gm. of protein was given. The urine volume immediately rose to 4900 and 5270 cc. There was a slight increase in the sodium chloride output on June 13, but the effect in general seems to represent a true diuresis from protein not explainable by changes in the sodium chloride output.

After sufficiently long observations on the 90 gm. protein intake, it had been intended to reduce the protein again to 30 gm., for the sake of comparison. This plan miscarried when it became necessary to discharge the patient on June 29. It was arranged, however, for him to proceed with his former low salt, low protein diet at home, and with this his urine volume has again fallen to the former low level and he has remained entirely comfortable.

TABLE 7. CASE 11.

Case No. 879.

PITUITRIN TEST.

June 17, 1922.

Time	URINE					Water Intake cc.
	Vol. cc.	Sp. gr.	NaCl %	Total NaCl gm.	Albumin	
7 to 11 A. M.	760	1006	.014	.1164	Faint	200
11 to 12	340	1006	.028	.0952	"	200
12 to 1	270	1005	.026	.0715	"	200
1 to 2	245	1006	.036	.0882	"	200
2 P. M.	0.3 cc	pituitary extract sprayed into nostrils.				
2 to 3	65	1010	.060	.0390	"	200
3 to 4	78	1010	.020	.0156	"	200
4 to 5	110	1008	.010	.0110	"	200
5 to 6	195	1007	.014	.0233	"	200
6 to 7	145	1007	.016	.0232	"	200
7 to 8	225	1007	.015	.0337	"	200
8 to 9	335	1007	.014	.0469	"	200
9 to 10	250	1007	.014	.0350	"	200
10 to 11	190	1006	.013	.0247	"	200
11 to 7 A. M.	560	1006	.014	.0784	"	200

Table 7 shows that pituitrin sprayed into the nostrils was effective in reducing the volume and slightly raising the specific gravity of the urine with a fixed water intake. Table 8 shows a more marked effect of pituitrin given subcutaneously. On account of the very low salt intake, it seems

improbable that these results of pituitrin were produced by any direct action upon sodium chloride metabolism. They conform rather to the general belief that pituitrin modifies kidney function.

TABLE 8. CASE 11.

Case No. 879.

PITUITRIN TEST.

June 21, 1922.

Time	URINE					Water Intake cc.
	Vol. cc.	Sp. gr.	NaCl %	Total NaCl gm.	Albumin	
6 to 7 A. M.	374	1006	.013	.048	V. Faint	200
7 to 8	304	1006	.012	.037	"	200
8 to 9	247	1006	.014	.034	"	200
9 to 10	234	1006	.011	.027	"	200
10 to 11	439	1006	.011	.051	"	200
11 to 12	394	1006	.015	.059	"	200
12 to 1	248	1007	.019	.049	"	200
1 to 2	184	1009	.012	.010	"	200
2 to 3	212	1008	.006	.013	"	200
3 P. M.	Given	$\frac{1}{2}$ cc. pituitrin by hypodermatically.				
3 to 4	75	1011	.020	.015	"	200
4 to 5	40	1018	.033	.013	"	200
5 to 6	44	1020	.036	.015	"	200
6 to 7	73	1015	.037	.027	"	200
7 to 8	143	1010	.020	.028	"	200
8 to 9	133	1009	.020	.026	"	200
9 to 10	115	1009	.033	.037	"	200
10 to 11	185	1008	.016	.029	"	200

TABLE 9. CASE 11.

Case No. 879.

PITUITARY EXTRACT AND GLUCOSE.

June 28, 1922.

Hour Period	BLOOD	URINE						Water Intake cc.
	Plasma Sugar mg. per 100 cc.	Vol. cc.	Sp. gr.	NaCl %	Total NaCl gm.	Albumin	Sugar	
8:35- 9:35	.....	250	1006	.016	.040	neg.	0	200
9:35-10:35	.....	335	1006	.016	.053	"	0	200
10:35-11:35	.....	275	1005	.014	.038	"	0	200
11:35-12:35	.....	220	1005	.018	.039	"	0	200
12:35- 1:35	.....	175	1007	.012	.021	"	0	200
1:35- 2:35	.....	245	1005	.014	.034	"	0	200
	0.4 cc. pituitrin by hypodermic, also,							
	100 gm. glucose by mouth.							
2:35- 3:35	157	90	1013	.020	.018	"	0	200
4:05-	334	.....	.....	.....	.....	.....	.....	.....
3:35- 4:35	341	30	1019	.031	.009	"	0	200
4:35- 5:35	199	55	1019	.018	.009	"	0	200
5:35- 6:35	.....	145	1009	.012	.017	"	0	200
6:35- 7:35	.....	140	1009	.010	.014	"	0	200
7:35- 8:35	129	215	1006	.008	.017	"	0	200
8:35- 9:35	.....	248	1006	.008	.019	"	0	200
9:35-10:35	.....	300	1006	.009	.027	"	0	200
10:35-7 A.M.	.....	1370	1005	.012	.164	.....	.....	.....

Table 9 shows the results of simultaneous administration of pituitrin subcutaneously and glucose by mouth. The usual reduction of volume and increase of gravity of the urine are evident. The tolerance was lowered by pituitrin, as indicated by the marked elevation of plasma sugar, but the kidneys were highly impermeable to glucose, so that none appeared in the urine.

### CASE III (No. 1112).

Male. American. Age 16. Schoolboy. Admitted April 3, 1922.

*Family History:* Mother living and well at 40. Father living and well at 59. One sister living and well. Two brothers, aged 25 and 18 years, are living and well.

*Past History:* Patient's health was always good. He had measles and whooping cough before the age of 9, but no other known diseases. Tonsillectomy 7 years ago. Appetite excellent. Extremely fond of sweets and starches. Never suffered from thirst or polyuria until the present illness. Has lived an active life in rural district. Always normal in weight. His present weight is 130 pounds. Height 5 ft. 4 in.

*Present Illness:* At the age of 11 he had otitis media, and the left ear discharged continuously for  $1\frac{1}{2}$  years. He occasionally had irregular temperatures, but never above 101 degrees. At no time was he confined to his bed on account of the condition. Three months after onset of otitis media he suddenly developed thirst. The patient is certain that thirst preceded polyuria. The 24-hour urine specimens were negative for sugar and albumin, and varied from a maximum volume of 4 gallons to a minimum of 3 gallons. For the first ten days his appetite was poor, and he ate irregularly, but since that time his appetite has been normal, and he has eaten liberally of all kinds of food.

The urine volume gradually decreased until at the end of the second year he was voiding only 2 gallons per day, and remained at this level until one year ago, when he again had otitis media, with purulent discharge. Polyuria increased to as much as  $5\frac{1}{2}$  gallons per day.

At no time has he been free from polyuria and thirst. Nocturia has been constant.

*Physical Examination:* Well nourished young male. Skin is normal in color, and there is no edema. Distinct acromegalic facies. Patient is partially deaf. The right auditory canal is normal in shape. The ear drum has lost its lustre. The left ear drum has been obliterated. There is no discharge. No evidence of active infection. Throat normal. Teeth in good condition. Heart and lungs normal. Abdomen negative. Extremities: the hands and feet are large in proportion to the general structure of the body. The hands are spade-like in appearance. Diagnosis: Diabetes insipidus.

*Laboratory Findings:* Urine volume, with minimum possible drinking, 10,430 cc. in 24 hours. Specific gravity 1007. Total NaCl 8.14 gm. Blood



TABLE 10. CASE III.

Case No. 1112 Date 1922	DIET					URINE				BLOOD				Weight, Lb.	
	Pro- tein gm.	Fat gm.	C. H. gm.	NaCl gm.	Total Cal.	Vol. cc.	Sp. gr.	Total NaCl gm.	T. N. gm.	Plasma Sugar mg. per 100	Plasma NaCl per 100	Blood Urea cc.	Red Cell Vol. %	A. M.	P. M.
Apr. 3	Unrestricted	food	and salt.												
4	"	"	"	"	"	10430	1007	8.14	15.58	143	589	20	.....	114	.....
5	"	"	"	"	"	10970	1004	5.37	15.83	.....	.....	.....	.....	114	.....
6	"	"	"	"	"	10290	1005	6.17	14.83	.....	.....	.....	.....	.....	.....
7	"	"	"	"	"	12150	1003	5.47	14.27	.....	.....	.....	.....	.....	.....
8	"	"	"	"	"	13890	1003	4.02	10.11	.....	.....	.....	.....	114	.....
9	80	148	210	20	2500	14350	1004	12.60	17.88	120	568	20	.....	117	117
10	"	"	"	20	"	17456	1003	23.70	10.99	.....	.....	.....	.....	117	120
11	"	"	"	20	"	17400	1004	14.26	13.88	.....	.....	.....	.....	118	118
12	"	"	"	20	"	15084	1004	12.37	11.61	.....	.....	.....	.....	118	116
13	"	"	"	20	"	16600	1003	15.10	11.38	123	601	33	50.5	117	115
14	"	"	"	0	"	11600	1003	11.02	11.36	.....	.....	.....	.....	119	119
15	"	"	"	0	"	9525	1003	10.19	11.33	.....	.....	.....	.....	.....	.....
16	"	"	"	0	"	6600	1004	2.17	11.18	.....	.....	.....	.....	114	.....
17	"	"	"	0	"	9300	1005	2.23	15.36	157	589	30	48.3	115	116
18	"	"	"	0	"	8490	1004	2.46	14.66	.....	.....	.....	.....	.....	.....
19	"	"	"	0	"	6450	1003	1.29	10.11	.....	.....	.....	.....	.....	.....
20	"	"	"	0	"	8444	1004	0.67	15.13	112	568	20	47.1	112	.....
21	160	113	210	0	"	10250	1004	0.41	16.50	.....	.....	.....	.....	111	.....
22	"	"	"	0	"	11430	1006	0.45	18.56	.....	.....	.....	.....	.....	.....
23	"	"	"	0	"	9215	1004	0.36	18.31	.....	.....	.....	.....	.....	.....
24	"	"	"	0	"	13050	1005	0.52	22.28	.....	.....	.....	.....	115	.....
25	"	"	"	0	"	12946	1005	1.55	23.38	160	577	16	52.1	113	117
26	"	"	"	0	"	12060	1005	2.41	23.97	.....	.....	.....	.....	113	.....
27	25	155	250	0	"	9040	1004	1.08	12.88	.....	.....	.....	.....	115	.....
28	"	"	"	0	"	10000	1004	0.80	8.68	.....	.....	.....	.....	.....	.....
29	"	"	"	0	"	5570	1003	0.66	.....	.....	.....	.....	.....	.....	.....
30	"	"	"	0	"	9785	1004	1.17	5.34	.....	.....	.....	.....	116	117
May 1	"	"	"	0	"	7440	1005	1.48	5.00	122	.....	.....	.....	115	117
2	"	"	"	0	"	4572	1003	0.90	3.09	585	.....	.....	.....	111	115
3	"	"	"	0	"	7650	1006	0.30	6.00	997	.....	.....	.....	115	.....
4	"	"	"	0	"	6380	1006	0.25	4.46	132	577	10	47.2	113	.....



urea 20 gm. per 100 cc. Plasma sugar 143 gm. per 100 cc. Plasma chloride 589 gm. per 100 cc. Wassermann negative.

X-ray examination by Dr. H. M. Imboden as follows: "The X-ray examination of the skull shows the vault of more than average thickness and density. The inner table is perfectly smooth. There are no irregularities to indicate increased pressure, neither are there unusual groovings for the meningeal vessels. The size of the sella compares favorably with that of the skull, and there are no erosions of any part."

### DISCUSSION OF CASE III.

Table 10 summarizes the clinical record in the Institute. Owing to moderate restriction of the salt intake at home, the urine volume at admission was only 10 liters. The patient also did not make use of his opportunity to eat salt freely, and up to April 8 he maintained a comparatively low chloride output. The fluid allowance was kept at the minimum permitted by thirst.

Beginning April 9, a weighed diet was instituted, and up to April 13 this included 20 gm. of sodium chloride per day. There was considerable salt retention; also, the increase of thirst and diuresis was not in proportion to the increased chloride output. Nevertheless, the influence of the salt in increasing the polyuria was very evident.

Beginning April 14, the diet was made salt-free. The urine volume fell very decidedly, while the retained salt was gradually eliminated. Nevertheless, the urine volume on April 20 was still 8444 cc.

April 21 to 26, the protein intake was doubled. The result was a distinct increase of thirst and diuresis, without appreciable change in the specific gravity. Though there was some tendency to sweeping out of salt, culminating in the excretion of 2.41 gm. on April 26, the result in general is not explainable by sodium chloride but must be regarded as a true protein diuresis.

Beginning April 27, the protein was sharply reduced to 25 gm. daily, while the total calories were kept at the same level by an increase of carbohydrate and fat. The urine volume was thus reduced to decidedly lower quantities than before. The specific gravity was not particularly changed.

The test with 10 gm. salt on May 20 resulted in marked polyuria, though the elimination of the extra chloride was prolonged over several days. An even greater increase of urine resulted from the test with the addition of 100 gm. protein on May 22. No reduction of the 24-hour urine volume resulted from the earlier pituitrin tests, the effects of which were ended within a few hours; but the repetition of the doses on May 31 resulted in a decided diminution of the polyuria on this date.

The red cell volume was high, as seems to be usual in these cases. The gradual fall which occurred under treatment is perhaps one sign of a return toward normal conditions.



Case III





*Case III*



*Case IV*





This case was the severest of the series, and was not thoroughly controlled by the stringent and prolonged limitations of salt and protein, inasmuch as the urine output remained approximately 5 liters per day. This result, however, represented a distinct clinical benefit, as the patient was relieved of the most distressing degrees of thirst and polyuria. From beginning to end, he was urged to drink as little as possible, but could not restrain his thirst to any greater extent than represented by the urine volume on the various diets. The restrictions of both salt and protein were fairly well borne, though there may have been a very slight reduction of strength. This patient was the only one of the series in whom any suspicion arose of any deleterious effect of the diet.

TABLE 11. CASE III.

## TOLERANCE TEST

With 100 gm. Glucose.

Case No. 1112

Water Intake 200 cc. Per Hour.

Time	BLOOD				URINE				
	Plasma Sugar mg.	Plasma NaCl per 100 c.	Urea c.	R. C. Vol. %	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl	Sugar
Before	112	570	14	42.0	181	1005	.024	.043	0
½ Hr. After	265	593	16	39.6	—	—	—	—	—
1 Hr. After	206	593	14	38.8	345	1004	.020	.069	0
2 Hrs. After	135	593	14	38.1	65	1006	.058	.037	0
3 Hrs. After	115	579	14	30.9	275	1004	.029	.079	0
4 Hrs. After	—	—	—	—	95	1004	.041	.038	0
5 Hrs. After	—	—	—	—	515	1003	.033	.169	0

The test with 100 gm. glucose showed a slightly reduced tolerance, as indicated by the plasma sugar, but there was no glycosuria.

TABLE 12. CASE III.

Case No. 1112.

## SALT TEST

May 20, 1922.

Time	BLOOD				URINE				Water Intake cc.
	Plasma Sugar mg.	Plasma NaCl per 100 c.	Urea c.	R. C. Vol. %	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl	
8 A. M.	—	—	—	—	345	1003	.016	.055	450
9 A. M.	—	—	—	—	420	1003	.020	.084	450
	10 gm. NaCl by mouth.								
10 A. M.	157	608	20	44.6	610	1003	.029	.176	450
11 A. M.	122	634	22	39.5	290	1001	.029	.084	450
12 M.	120	639	22	45.8	470	1001	.058	.272	450
1 P. M.	120	639	28	42.1	665	1005	.098	.631	450
2 P. M.	113	667	26	41.8	360	1005	.095	.347	450
3 P. M.	109	631	24	40.8	370	1003	.086	.318	450
4 P. M.	112	635	24	42.5	480	1004	.086	.413	450

Table 12 shows the results of a single dose of 10 gm. of sodium chloride, after the patient had become adjusted to salt-free diet. The blood analyses before the taking of the salt on this day were unfortunately lost, but assuming that they were similar to those shown in Table 10 for all the preceding and following days, it is evident that the salt ingestion was followed by a prompt and marked increase of the chloride concentration of the plasma. The excretion in the urine was also decidedly increased, through an increase of both the urine volume and its chloride percentage. All three reached their maximum together at 1 P. M., when the volume was 665 cc. and the chloride concentration was 0.098 per cent. Also at nearly the same time (2 P. M.) the plasma chloride reached its highest level of 0.667 per cent. Though the urinary chloride concentration remained far below that which is known to be possible to the normal kidney, it is doubtful if higher concentration would be shown by a normal person drinking 450 cc. of water per hour. The experiment seems to indicate a distinct power of the kidneys to alter the concentration of sodium chloride in this severe case of diabetes insipidus. Also, as the chloride percentage of the urine was so far below that of the blood, the polyuria cannot be explained as a mere hyposthenuria.

TABLE 13. CASE 111.

Case No. 1112.

## PROTEIN TEST

May 22, 1922.

Time	BLOOD			URINE					Water Intake cc.
	Plasma Sugar mg.	Plasma NaCl per 100	Urea cc.	Vol. cc.	Sp. Gr.	Urea mg. per 100 cc.	NaCl %	Total NaCl gm.	
6 A. M.	.....	.....	....	320	1005	120	.029	.092	450
7 A. M.	.....	.....	....	485	1003	96	.029	.140	450
8 A. M.	.....	.....	....	430	1005	108	.049	.210	450
9 A. M.	100	593	16	610	1004	72	.058	.353	450
10 A. M.	123	593	16	665	1003	60	.074	.492	450
11 A. M.	111	612	17	500	1003	81	.099	.495	450
12 M.	.....	.....	....	455	1004	132	.086	.391	450
1 P. M.	107	616	22	370	1005	204	.074	.273	450
2 P. M.	.....	.....	....	322	1006	273	.066	.212	450
3 P. M.	124	618	27	555	1005	240	.069	.382	450
4 P. M.	.....	.....	....	370	1006	345	.058	.214	450
5 P. M.	112	626	40	275	1005	432	.037	.101	450
May 23, 1922	.....	.....	....	.....	.....	.....	.....	.....	.....
11 A. M.	.....	.....	22	.....	.....	.....	.....	.....	.....

Table 13 shows the result of a single meal of 100 gm. protein at a time when the patient had become accustomed to a ration of only 25 gm. protein. Distinct diuresis of both water and sodium chloride resulted, notwithstanding the fixed and liberal water intake of 450 cc. per hour. This result may possibly have been modified by the salt test given 2 days before, which provided a salt reserve which could be drawn upon on such an occasion. The plasma chloride seemed to rise more promptly than the blood urea, though both reached their maximum at 5 P. M. The specific gravity of the urine rose only slightly, but the urea concentration increased greatly and likewise reached its maximum at 5 P. M. The ability of the kidneys to concentrate urea is thus demonstrated, and it is doubtful if any higher concentration would be shown by a normal person drinking 450 cc. of water per hour.

TABLE 14. CASE III.

Case No. 1112.

PITUITRIN TEST

May 27, 1922

Time	BLOOD				URINE					Water Intake cc.
	Plasma Sugar mg.	Plasma NaCl per 100	Blood Urea cc.	R. C. V. %	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl	Urea mg. per 100 cc.	
8:45	---	---	---	---	---	---	---	---	---	---
9:45	---	---	---	---	400	1005	.029	.116	170	450
10:45	---	---	---	---	400	1005	.020	.080	54	450
11:45	---	---	---	---	305	1003	.024	.073	72	450
12:45	---	---	---	---	495	1004	.020	.099	72	450
1:45	---	---	---	---	327	1003	.020	.065	120	450
2:45	159	575	12	42.5	330	1003	.029	.095	204	450
2:45	$\frac{1}{2}$ cc. pituitrin hypodermatically.				---	---	---	---	---	---
3:45	130	558	16	39.1	63	1005	.037	.022	204	450
4:45	---	---	---	---	23	1015	.091	.020	384	450
5:45	192	506	14	37.5	33	1012	.070	.023	516	450
6:45	---	---	---	---	98	1007	.033	.032	204	450
7:45	---	---	---	---	140	1007	---	---	---	450
8:45	---	---	---	---	200	1006	.015	.030	---	450
9:45	---	---	---	---	305	1004	.012	.036	228	450
10:45	---	---	---	---	295	1005	.012	.035	216	450
11:45	---	---	---	---	295	1006	.008	.023	96	450
12:45	---	---	---	---	390	1005	.012	.046	96	450
12:45 to 7:00	---	---	---	---	2621	---	---	---	---	---

Table 14 shows the usual effect of a small dose of pituitrin subcutaneously in reducing the urine volume and raising the specific gravity with a fixed water intake. The fall of red cell volume indicated slight dilution of the blood by the retained water.

Table 15 shows the hourly observations of the urine on May 28, without therapeutic intervention, and with permission to the patient to drink strictly according to thirst.

TABLE 15. CASE III.

Case No. 1112

May 28, 1922.

Time	BLOOD				URINE					Water Intake
	Plasma Sugar mg.	Plasma NaCl per 100	Blood Urea cc.	R. C. Vol. %	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl	Urea mg. per 100 cc.	
11:45	142	547	14	41.2	-----	-----	-----	-----	-----	Water intake not regulated or restricted
1:00	-----	-----	-----	-----	245	1006	.037	.090	-----	
2:00	-----	-----	-----	-----	215	1006	.029	.057	-----	
3:00	-----	-----	-----	-----	205	1006	.033	.067	-----	
4:00	-----	-----	-----	-----	255	1008	.033	.084	-----	
5:00	-----	-----	-----	-----	245	1009	.041	.100	-----	
6:00	-----	-----	-----	-----	315	1008	.020	.063	-----	

TABLE 16. CASE III.

## PITUITRIN TEST

Case No. 1112.

(Fluid intake not regulated.)

May 30, 1922.

Time		URINE			
		Vol. cc.	Sp. Gr.	NaCl %	Total NaCl
11 to 12	A.M.	395	1003	.029	.114
12 to 1	P.M.	330	1003	.033	.099
1:00	"	Nares	sprayed with	0.2 cc. pituitrin.	
1 to 2	"	95	1003	.033	.032
2 to 3	"	105	1004	.033	.034
3 to 4	"	70	1009	.047	.032
4 to 5	"	175	1003	.020	.035
5 to 6	"	175	1002	.020	.035
6 to 7	"	145	1004	.016	.023
7 to 8	"	160	1005	.016	.025
8 to 9	"	120	1003	.012	.014
9 to 10	"	150	1001	.014	.021
10 to 11	"	205	1004	.012	.024
7:30 A. M. to 11 A. M. and 11 P. M. to 7 A. M.		3300	1004	.029	-----

Table 16 shows definite effects from spraying the nostrils with only 0.2 cc. of pituitrin.

TABLE 17. CASE III.  
PITUITRIN TEST.

May 31, 1922.

PITUITRIN TEST.												
Time	BLOOD					URINE						
	Plasma Sugar mg.	Plasma NaCl per 100	Blood Urea cc.	R. C. V. %	Hgb. %	Vol. cc.	Sp. Gr.	NaCl %	Total NaCl	Albumin gm. per liter	Urea mg. per 100 cc.	Water Intake cc.
10 A. M.	.....	.....	.....	40.6	100	275	1003	.029	.079	0	110.5	200
to 11	150	593	22	.....	.....	372	1001	.020	.074	0	89.25	200
11:00	Nostrils sprayed with 0.3 cc. pituitrin.											
to 12	.....	.....	.....	.....	.....	158	1001	.029	.045	.2	127.5	200
to 1 P. M.	.....	.....	.....	.....	.....	82	1003	.055	.045	.3	204.	200
to 2	.....	.....	.....	.....	.....	184	1003	.033	.060	.3	153.	200
to 3	.....	.....	.....	.....	.....	195	1002	.026	.050	.3	153.	200
to 4	.....	.....	.....	.....	.....	125	1004	.029	.036	.....	246.8	200
to 5	.....	.....	.....	.....	.....	85	1004	.016	.013	.3	192.	200
to 6	.....	.....	.....	.....	.....	205	1004	.016	.032	.....	120.	200
to 7	125	.....	.....	.....	.....	86	1006	.022	.....	.4	216.	200
7:00	Nostrils sprayed with 0.3 cc. pituitrin.											
to 8	125	589	18	40.4	93.7	65	1012	.041	.026	1.5	359.	200
to 9	.....	.....	.....	.....	.....	77	1011	.041	.009	1.7	504.	200
to 10	.....	.....	.....	.....	.....	56	1011	.033	.014	1.4	168.	200

Case No. 1112.



Table 17 shows the results of spraying the nostrils with pituitrin twice on the same day. The urine volume was greatly reduced, notwithstanding the fixed intake of 200 cc. water per hour. The chloride concentration and the specific gravity were not greatly altered, but the urea concentration was decidedly increased. The red cell volume was not apparently changed, but the fall of hemoglobin may indicate a dilution of the blood. Albumin, which had been absent from the urine, appeared with the first dose of pituitrin and increased with the second dose.

Unless it be assumed that the tissues of the diabetes insipidus patient are abnormally dried, the suppression of urine by pituitrin with a fixed water intake must be regarded as an abnormality and not a simple restoration of a physiological function. The appearance of albuminuria seems to give a similar indication.

#### CASE IV. (No. 1311).

Male. American. Single. Age 48. Forest supervisor. Admitted Jan. 13, 1923.

*Family History:* Mother living and well at 60. Father died at 51 of pneumonia. Two brothers living and well. One sister living and well. There is no history of diabetes mellitus or diabetes insipidus in the family.

*Past History:* Always healthy and strong. Had measles, mumps and whooping cough when a child. Never suffered from headaches, dizziness, shortness of breath or abdominal distress. In 1898 he enlisted in the Spanish-American War, and while stationed in Florida had malaria and acute yellow jaundice. Took large doses of quinine for several months. In 1899 had a luetic infection, with hard chancre on the penis, followed by a rash which covered the whole body. He has received luetic treatment on four occasions. Wassermann tests made in 1915, and again in 1918, were negative. In 1915 he received three injections of salvarsan.

*Present Illness:* The onset of diabetes insipidus was in October, 1905. The first thing he noticed was extreme thirst, followed by polyuria. He attributes the onset of the disease to his sudden exposure to cold. He assisted in rescuing a woman from a river in which there was much ice and snow. He was exposed to the cold water for about fifteen minutes, and the following day the symptoms of the disease appeared, and continued for three days. On the fourth day he became drowsy and mentally hazy. His sleep was greatly disturbed, on account of nocturia. He lost his appetite and on several occasions food was repulsive, and he would vomit after eating. During the first four months of the disease the urine volume ranged from 12 to 16 quarts per day.

Since 1905 he has suffered constantly from thirst, and has always passed large quantities of urine. He is certain that his condition is gradually getting better year after year, but at no time has there been a complete remission. The smallest amount of urine passed during the 24 hours has been 6 quarts. On several occasions he has passed as much as 16 quarts, over brief periods ranging from 1 to 4 months.

In July, 1916, he began to notice puffiness of the ankles and thighs. After a day of heavy work or horseback riding the ankles would become edematous, and he could easily make pits in the skin by pressure. He has never suffered from shortness of breath, palpitation or visual changes. The skin has always been dry and somewhat yellow.

Only on one occasion has he taken drugs, and that in the beginning, when he was given tincture of valerian. He felt that it was of little value, and discontinued its use after one month. He has never been advised to alter the diet, but has continued to take normal amounts of protein, carbohydrate, fat, condiments and salt in whatever quantities his appetite demanded. He has never used alcohol. On several occasions he has been in regions where the water contained large quantities of lime and alkali, and has found that his thirst increased markedly, and that it was difficult to get enough water to relieve it.

The present body weight is 200 pounds. It has averaged 196 pounds for the past 12 years.

*Physical Examination:* Heavy-set, well developed adult male, with a tendency to obesity. The skin of the face and cheeks is highly complexioned, but the skin of the body is slightly yellow and pasty. There is very slight edema of the ankles, which pits on pressure. The patient is not dyspneic, and has no complaints except of thirst. The head is normal in shape. The eyes react normally to light and accommodation. Eye-grounds normal. Thorax—antero-posterior diameter increased. There is no impairment or dullness on percussion. No rales. Heart is normal in outline. No murmurs. Blood pressure 125/70. The abdomen is covered with a thick layer of adipose tissue, and is distended considerably with gas. Liver and spleen not palpable. Liver outline normal on percussion.

*Extremities:* Glandular system normal. Reflexes present and equal. There are four copper-colored spots over the right and left tibiae in front.

*Laboratory Findings:* Wassermann negative. Blood urea 16 mg. per 100 cc. Plasma chloride 606 mg. per 100 cc. Plasma sugar 115 mg. per 100 cc. Red cell volume 50 per cent. Hemoglobin 80 per cent. Red cell count 4,348,000.

*Urine:* Pale, almost colorless. Neutral reaction. Specific gravity 1010. NaCl percentage 0.181. Albumin, very faint trace. No sugar and no acetone. Diagnosis: Diabetes insipidus. Tertiary lues.

#### DISCUSSION OF CASE IV.

Upon admission, the patient was allowed to choose his entire diet at will, and was asked especially to imitate his former habits regarding protein and salt as closely as possible. On this program he excreted approximately 8 liters of urine, 8 gm. of sodium chloride and 12 gm. of nitrogen. The results were not greatly changed by the weighed diet of Jan. 15, which included 90 gm. protein and 6 gm. salt.

TABLE 18. CASE IV.

Case No. 1311

Date 1923	DIET					URINE				BLOOD			Weight Lb.
	Protein gm.	Fat gm.	C. H. gm.	NaCl gm.	Total Cal.	Vol. cc.	Sp. Gr.	Total NaCl gm.	T. N.	Plasma Sugar mg.	Plasma NaCl per 100	Blood Urea cc.	
Jan. 13	Unweighed diet.												
14	"	"	"			7805	1010	8.35	12.09	115	606	16	200
15	90	166	160	6	2500	7600	1006	9.61	14.89	130	603	18	200
16	90	166	160	0	2500	6575	1008	6.37	11.04				200
17	90	166	160	0	2500	6100	1007	4.51	13.83				200
18	90	166	160	0	2500	4900	1009	2.59	13.6	104	601	20	199
19	90	166	160	0	2500	4550		2.05	12.35				200
20	90	166	160	0	2500					127	583	18	200
21	90	166	160	0	2500		1009						200
22	90	166	160	0	2500	4500	1008	1.84	11.08	113			200
23	90	166	160	0	2500	5100	1007	1.78	15.13				200
24	90	166	160	0	2500	4600	1008	1.88	18.19				200
25	20	197	160	0	2500	3700	1010	1.53	9.77	126	583	18	202
26	20	197	160	0	2500	3200	1005	2.62	5.10				202
27	20	197	160	0	2500	2700	1004	.891	5.67				201
28	20	197	160	0	2500	3080	1005						201
29	20	157	250	0	2500	2050	1009	1.59	15.42				201
30	20	157	250	0	2500	1610	1008			182	566	12	201
31	20	157	250	0	2500	1740	1005	1.14					



Table 20 shows the usual effects of a small dose of pituitrin subcutaneously in reducing the urine volume and raising the specific gravity. It may be remarked that the ordinary specific gravity, as shown in Table 18, was higher than in the average diabetes insipidus case. As the theory that diabetes insipidus consists in an inability of the kidneys to concentrate urine is now practically discredited, this peculiarity need not cast doubt upon the diagnosis in this case. The renal or vascular lesion which was responsible for slight edema in previous years may also have been due to syphilis; but, even if a slight nephritis be admitted as a complication in spite of the normal kidney function tests, diabetes insipidus of syphilitic origin must still be considered the most probable cause of the excessive thirst and polyuria.

The trace of albuminuria which frequently follows pituitrin dosage was also evident in this case.

### CONCLUSIONS.

1. These observations have given further examples of a number of points already familiar in the literature of diabetes insipidus. The cause was most probably syphilis in Case IV and middle-ear infection in Case III, though the relationship with the hypophysis was merely suggestive. The etiology was unknown in the other two cases. Bradyuria, perhaps in connection with slow absorption, was apparent in certain tests in Case II but not in the others. The plasma chloride concentration was low in Case II, but was above normal in the other two cases examined, particularly in view of the low chloride intake. The red cell volume was high in all three cases examined, though the hemoglobin and red cell counts were comparatively low. These tests are too few and inadequate to permit any conclusion whether the water content of the blood or tissues is reduced in connection with the abnormal thirst and diuresis.

2. Pituitrin, given either subcutaneously or by nasal spray, showed its usual effect in reducing thirst and polyuria and raising the concentration of the urine. Water retention thus occurred when the intake was constant, and albuminuria was often produced, even with small doses. As pituitrin is known to reduce the urine similarly in normal persons or animals, it is still a question whether these results do not represent a mere drug action rather than a true hormonal substitution.

3. The essential disorder did not consist in a loss of concentrating power on the part of the kidneys, according to the following evidence: (a) there was no important retention of either



chloride or urea in the blood after test feedings of salt or protein, even when the excretion in some instances was considerably delayed; (b) the abnormal thirst and diuresis sometimes continued with dietary restrictions such as control the polydipsia and polyuria of even the severest nephritic cases; (c) a very considerable concentrating power was demonstrable by direct tests, especially for urea; (d) sodium chloride acted as a diuretic even when its percentage in the urine was far lower than in the plasma.

4. Protein feeding has caused a flushing out of chlorides in the cases studied, but a direct increase of water diuresis due to the increase of nitrogen is also evident. Reduction of thirst and urine within convenient limits without any close limitation of protein was possible only in the mildest of these four cases (Case I). The other three required more or less radical restriction of protein for their successful treatment.

5. Restriction of sodium chloride was essential in the treatment of all four cases. Two points were established in regard to it: (a) as the diuresis is not necessarily in direct ratio with the quantity of chloride, it is not possible to ignore small quantities of salt, but the fullest results are obtainable only with continuous reduction of NaCl in the urine to a fraction of a gram per day, as in hypertension cases; (b) except for slight doubtful symptoms in Case III, the stringent chloride privation was borne by all four of these patients without the slightest sign of deleterious effect. Diabetes insipidus patients therefore resemble the majority of hypertension patients in being able to endure indefinitely salt restriction of a degree which soon causes serious disturbance in normal persons.

6. These restrictions of both protein and salt are not difficult to apply with proper culinary art, and experience has indicated that the effort is well worth while. In the most severe of these cases the urine volume was reduced to approximately five liters, and the other three patients were made completely comfortable. As usual, diet is only palliative, in the sense of relieving a damaged function, but it is the most important practical treatment for the great majority of cases of diabetes insipidus.



## REFERENCES.

1. Allen, F. M., and Sherrill, J. W. *J. Metabolic Research*, 2, 1923, 429-545. The treatment of arterial hypertension.
2. Bailey, P., and Bremer, F. *Arch. Int. Med.*, 28, 1921, 773-803. Experimental diabetes insipidus.
3. Bauer, J., and Aschner, B. *Wiener Archiv für innere Medizin*, 1, 1920, 297-334. Die Pathogenese des Diabetes insipidus.
4. Bergé, A., and Schulman, E. *Presse méd.*, Dec. 5, 1918. (Abstr. Practical Medicine Series, Vol. 1, *General Medicine*, 393-394. Hypophyseal polyuria).
5. Berglund, H. *Studier över Koksaltomsättningens; Fysiologi och Patologi*. Stockholm, 1920.
6. Blumgart, H. L. *Arch. Int. Med.*, 29, 1922, 508-514. The antidiuretic effect of pituitary extract applied intranasally in a case of diabetes insipidus.
7. Bullowa, J. C. M. *Med. Record*, 93, 1918, 127. Observations on the treatment of diabetes insipidus with infundin.
8. Christie, C. D., and Stewart, G. N. *Arch. Int. Med.*, 29, 1922, 555-566. Study of some cases of diabetes insipidus with special reference to the detection of changes in the blood when water is taken or withheld.
9. Ellern, H. *Dtsch. Arch. klin. Med.*, 109, 1912-13, 85-111. Ein Beitrag zum ätiologischen Studium des Diabetes insipidus.
10. Engel, K. *Ztschr. klin. Med.*, 67, 1909, 112-130. Ueber Diabetes insipidus.
11. Finkelnburg, R. *Dtschr. Arch. klin. Med.*, 91, 1907, 345-377. Klinische und experimentelle Untersuchungen über Diabetes insipidus.
12. Finkelnburg, R. *Dtsch. Arch. klin. Med.*, 100, 1910, 33-51. Ueber das Konzentrationsvermögen der Niere bei Diabetes insipidus nach organischen Hirnerkrankungen.
13. Fitz, R. *Arch. Int. Med.*, 14, 1914, 706-721. A case of diabetes insipidus.
14. Forschbach. *Ztschr. klin. Med.*, 77, 1913, 151-159. Zur Frage des Konzentriervermögens der Niere beim Diabetes insipidus.
15. Forschbach and Weber. *Ztschr. klin. Med.*, 73, 1911, 221-239. Beobachtungen über die Harn-und Salz-Ausscheidung im Diabetes insipidus.
16. Futcher, T. B. *Modern Medicine, Its Theory and Practice*. Vol. 2, Chapter 17, 721-728. Diabetes insipidus.
17. Geigel, R. *Dtsch. Arch. klin. Med.*, 37, 1885, 51-58. Beiträge zur Lehre vom Diabetes insipidus.
18. Grote, L. R. *Dtsch. Arch. klin. Med.*, 122, 1917, 223-240. Ueber die Funktion der Niere bei Diabetes insipidus.
19. Hecht, E. *Ztschr. klin. Med.*, 90, 1920-21, 126-145. Zum Wesen des Diabetes insipidus.

20. Kennaway, E. L., and Mottram, J. C. *Quart. J. Med.*, 12, 1919, 225-258. Observations upon two cases of diabetes insipidus; with an account of the literature relating to an association between the pituitary gland and this disease.
21. Leschke, E. *Ztschr. klin. Med.*, 87, 1919, 201-279. Beiträge zur klinischen Pathologie des Zwischenhirns. I. Klinische und experimentelle Untersuchungen über Diabetes insipidus, seine Beziehungen zur Hypophyse und zum Zwischenhirn.
22. Meyer, E. *Dtsch. Arch. klin. Med.*, 83, 1905, 1-70. Ueber Diabetes insipidus and andere Polyurien.
23. Meyer, E. *Ztschr. klin. Med.*, 74, 1911-12, 352-354. Bemerkungen zu der Arbeit von Forschbach und Weber: Beobachtungen über die Harn und Salz-Ausscheidung im Diabetes insipidus.
24. Meyer, E., and Meyer-Bisch, R. *Dtsch. Arch. klin. Med.*, 137, 1921, 225-233. Beitrag zur Lehre vom Diabetes insipidus.
25. Minkowski, O. *Therap. d. Gegenwart*, 51, 1910, 4-8. Zur Therapie des Diabetes insipidus.
26. Motzfeldt, K. *Norsk Magazin for Laegevidenskaben*, 76, 1915, 1305-1376. Hypofyse ag diurese.
27. Oehme, C., and Oehme, Margaret. *Dtsch. Arch. klin. Med.*, 127, 1918, 261-299. Zur Lehre vom Diabetes insipidus.
28. Palmer, W. W. *Nelson Loose-Leaf Medicine*, 1920, Vol. 3, Chapter 4, 50-54. Diabetes insipidus.
29. Rabinowitch, I. M. *Arch. Int. Med.*, 28, 1921, 355-366. Metabolic studies on a case of diabetes insipidus.
30. Rees, M. H., and Olmstead, W. H. *Endocrinology*, 6, 1922, 230-234. The use of pituitary extracts by mouth in the treatment of diabetes insipidus.
31. Rowntree, L. G. *The Oxford Medicine*, 1921, Vol. 4, Chapter 6, 179-193. Diabetes insipidus.
32. Seiler, F. *Ztschr. klin. Med.*, 61, 1907, 1-31. Ueber das Wesen des Diabetes insipidus.
33. Stenström, T. *Endocrinology*, 6, 1922, 365-382. Diabetes insipidus; its pathogenesis and therapeutics.
34. Strauss, H. *Ztschr. exp. Path. u. Ther.*, 1, 1904-05, 408-418. Zur Kenntnis des Wasserstoffwechsels bei Diabetes insipidus.
35. Tallquist, T. W. *Ztschr. klin. Med.*, 49, 1903, 181-192. Untersuchungen über einen Fall von Diabetes insipidus.
36. Tucker, J. *Amer. J. Med. Sci.*, 163, 1922, 668-675. Immediate recovery from early diabetes insipidus after lumbar puncture. Report of a case.
37. Umber, F. *Ernährung und Stoffwechselkrankheiten*, 2nd Ed., Urban & Schwarzenberg, 1914. Chapter VII, Diabetes insipidus, pp. 314-334.
38. Veil, W. H. *Dtsch. Arch. klin. Med.*, 119, 1916, 376-436. Ueber die Wirkung gesteigerter Wasserzufuhr auf Blutzusammensetzung und Wasserbilanz. Beitrag zur Kenntnis der Polydipsie und des Diabetes insipidus.

39. Veil, W. H. *Biochem. Ztschr.*, 91, 1918, 267-316. Ueber die Bedeutung intermediärer Veränderungen im Chlorstoffwechsel beim Normalen und beim Nierenkranken.
40. Veil, W. H. *Biochem. Ztschr.*, 91, 1918, 317-389. Ueber intermediäre Vorgänge beim diabetes insipidus und ihre Bedeutung für die Kenntniss vom Wesen dieses Leidens.
41. Weir, J. F., Larson, E. E., and Rowntree, L. G. *Arch. Int. Med.*, 29, 1922, 306-330. Studies in diabetes insipidus, water balance, and water intoxication.





STUDIES CONCERNING THE INFLUENCE OF A  
DISTURBANCE IN THE ACID-BASE EQUILIBRIUM  
OF THE BLOOD ON RENAL FUNCTION  
AND PATHOLOGY\*

STUDY 1. THE EFFECT OF ACID AND ALKALINE SOLU-  
TIONS ON RENAL FUNCTION AND PATHOLOGY  
IN NORMAL DOGS.

Wm. deB. MacNider

*The Laboratory of Pharmacology, University of North Carolina.*

INTRODUCTION

The following studies are not primarily concerned with the production of renal injuries by the use of acid and alkaline solutions in the sense of obtaining nephropathic processes which are comparable to injuries which may be induced experimentally in animals by various nephrotoxic substances, and which occur in man from a variety of causes. The main interest in the different series of experiments has been to observe the functional and pathological response of the kidneys when these organs were furnished a blood of altered chemical composition by the introduction of an acid or an alkaline solution.

The ability of cells to functionate in a normal manner is profoundly influenced by the proper balance between hydrogen and hydroxyl ions in the fluid which bathes the cells and which gives to them their ever changing physico-chemical environment.

L. J. Henderson<sup>1</sup> in one of his numerous contributions to this field of investigation has stated so clearly the importance of the physico-chemical environment of cells that I take the liberty of quoting one of his statements. "The right working of physiological processes depends upon an accurate adjustment and preservation of physico-chemical conditions within the body. Throughout the animal body, while life exists, there occurs a regular formation of acid substances, excretory products of metabolism. Through resulting changes in equilibria between bases and acids, normal metabolism steadily operates to lower the inverting alkaline (neutral) reaction of the body. Like temperature and osmotic

---

\*Aided by a grant from the Rockefeller Institute for Medical Research.



pressure, the neutrality or alkalinity is adjusted by a mechanism within the body, but permanently maintained by exchanges with the environment."

In the studies which are to follow, the kidney has been selected as the organ in which to observe the influence of changes in this acid-base balance of the blood on its function and pathology for two reasons. In the first place the product of the kidney's functional response can be easily obtained and its composition determined. The kidney, unlike such organs as the liver, lends itself to a variety of fairly accurate functional tests which can be easily performed. Secondly; one of the essential functions of the kidney in health, as well as under the strain of certain diseased states, is to maintain either by elimination or retention of hydrogen and hydroxyl ions such a balanced neutrality of the blood that the kidney as well as other groups of cells may have a proper physico-chemical environment in which to functionate in a normal manner. For this latter reason in particular, it is of interest to study the functional and pathological response of the normal and previously diseased kidney when that physico-chemical balance of the blood which it so largely maintains at a point of neutrality is disturbed by the introduction of an acid or an alkaline solution.

The following investigation is divided into three parts. The first study is concerned with the influence of acid and alkaline solutions on renal function and pathology in normal animals. The second part deals with the influence of acid and alkaline solutions of the same strength on renal function and pathology in naturally nephropathic animals. The third part of the study is concerned with the ability of an alkaline solution to protect both the normal and naturally nephropathic kidney against injury from an acid solution.

The literature dealing with the effect of acids and alkalis when introduced into organisms has been mainly concerned with the metabolic disturbance induced by such injections, and with the way in which the disturbance which takes place in the acid-base equilibrium of the blood is readjusted. Only incidental attention has been paid to the influence of such solutions on renal function and pathology.

In a study concerning the alkalescence of the blood, Lassar<sup>2</sup> observed by crude methods of determination, a decrease in the alkalinity of the blood in dogs that had received an acid intravenously. In 1877, Walter<sup>3</sup>, in a research concerning the action of acids on the animal organism, made

the observation that in animals killed by the injection of acid into the blood, the blood apparently remained alkaline. He noted a depletion in the bases of the blood as shown by a decrease in the carbon dioxide tension, and furthermore made the observation of an increase in ammonia in the urine. The animals succumbed from a respiratory type of death which could be delayed by the use of sodium carbonate.

Loeb<sup>4</sup> in 1898 pointed out that whenever oxidations are impaired in tissues, the osmotic pressure rises, and ascribes this to an accumulation of incompletely oxidized metabolic products, particularly acids. He observed that under such conditions muscle tissue took up water and became edematous. In later publications<sup>5, 6</sup> Loeb has shown the importance of carbonates in the maintenance of the neutrality of tissue fluids and the media surrounding living cells.

Hoppe-Seyler and Araki<sup>7</sup> in one of the early and important papers on cell oxidations, made the observation that an interference with such processes leads to the abnormal production and accumulation of acids.

In the same year Hofmeister<sup>8</sup> demonstrated the influence on colloidal swelling of variations in the concentration of hydrogen and hydroxyl ions.

Some years later than these observations the studies of Fischer<sup>9, 10</sup> on edema and nephritis provoked much clinical interest and led to such critical analyses of the importance of a disturbance in the acid-base equilibrium of the organism as a factor in inducing edema and nephritis as are found in the study by Henderson, Palmer and Newburgh.<sup>11</sup>

The more recent literature has been concerned with the effect of acid and alkaline solutions on metabolism and to a less extent with the ability of such changes in the blood as these solutions induce to injure the kidney.

In 1918 Bornstein and Lippmann<sup>12</sup> suggested that the products of vigorous metabolism occurring in swimmers and in men subjected to heavy marching were a cause of certain transitory albuminurias. They noted a striking parallelism between the output of albumin and the presence of cylindroids in the urine with the acidity of the urine. The presence of both albumin and cylindroids was checked by the use of an alkali.

Fitz, Alsberg and Henderson<sup>13</sup> in a study of the excretion of phosphoric acid during an experimental acidosis in rabbits induced by giving hydrochloric acid, demonstrated that phosphates, as the mono and dipotassium phosphate, constituted a nearly neutral solution which had the property of taking up large quantities of acid or alkali. They concluded that slight changes in hydrogen ionization can hardly be without influence on the catalytic reactions of protoplasm.

Nagayama<sup>14</sup>, in his studies on renal activity and the acid-base equilibrium, observed the urea-excreting activity of the kidney to be less after administering an acid phosphate than after a neutral phosphate of the same phosphorus content. He concludes that the decrease in the alkalinity

of the plasma decreases the urea-excreting function of the kidney. Alkaline phosphates in equivalent amount to the acid phosphate had no appreciable effect.

The influence of acid and alkaline solutions on protoplasm have been studied in even more fundamental relations than their effect on meta-Sollmann's laboratory, an attempt was made to maintain the animal's concentration and the oxygen content of water upon tadpoles, Jewell<sup>12</sup> observed that the optimum hydrogen ion concentration approximates neutrality. Variations from this in either direction, as well as low temperature and low oxygen content of the water, progressively decreased both the rate and total amount of regeneration in tadpoles.

In two studies<sup>16, 17</sup> from this laboratory, it has been shown that the toxicity of uranium nitrate, which induces in part its injurious effect through the establishment of a disturbance in the acid-base equilibrium of the blood, is in large measure dependent upon the age of the organism in which a given degree of disturbance in this balance is induced. The tissues of old animals are more susceptible to such a disturbance than are the tissues of young animals. At a later date McArthur<sup>18</sup> confirmed these observations by demonstrating in planarians a decrease in the tolerance to acid and alkaline solutions as the age of the organism increased.

The following investigations have been undertaken to study the functional and pathological response not of the organism as a whole, but of an easily accessible organ, namely the kidney, to a disturbance in its physico-chemical environment induced by the introduction of acid and alkaline solutions into the blood stream.

## STUDY 1.

### *The Effect of Acid and Alkaline Solutions on Renal Function and Pathology in Normal Dogs.*

#### TECHNIQUE OF EXPERIMENTS.

Thirty-eight normal dogs were used in this series of experiments. Eighteen of these animals were used in the first part of the study and were given intravenously a solution of hydrochloric acid. Six of the animals were employed as controls and were not subjected to the action of the acid solution. The remaining eighteen animals of the series were used in the second part of the investigation and, with the exception of six animals that were reserved for control experiments, were given intravenously an alkaline solution.

The observations on these animals prior to any experimental interference has been similar to that described in previous publications. The dogs were kept in metabolism cages for four days before the day of experiment and were fed on lean raw beef and bread made from corn meal. Two hundred and fifty c.c. of water was given twice a day by

stomach tube. The urine was collected twice a day and subjected to the routine qualitative analysis for albumin, glucose and diacetic acid. Microscopic examinations were made each day from catheterized specimens. Two days before the day of experiment the functional response of the kidney was determined by the phenolsulphonephthalein test as conducted by Rowntree and Geraghty.<sup>19</sup> The elimination of the dye was estimated for a two hour period. For four days prior to the experiments the reserve alkali of the blood was determined by the method of Marriott.<sup>20</sup>

The technique employed in the experiments was as follows: Three hours before commencing an experiment the animal was given 250 c. c. of water by stomach tube. At the end of this period the animals were anesthetized by Gréchant's\* anesthetic in 60 per cent. strength. An hour was allowed for the development of a satisfactory state of anesthesia to permit the necessary operative interference. The animals were placed in a wooden operating rack which fits into the concavity of a copper box containing hot water. By such a device which was first employed in Sollmann's laboratory, an attempt was made to maintain the animal's normal body temperature during the period of the experiment. A tracheal canula was introduced for use in case it became necessary to employ either during the anesthesia. The carotid artery was exposed, a canula tied in place and connected in the usual manner to a mercury manometer. The femoral vein was exposed, a canula tied in place and connected with a burette. Through this connection the acid or alkaline solutions were introduced into the animals. The abdomen was opened through a small midline incision and canulas tied into each ureter. Urine flow was determined in drops per minute.

During the course of the experiments samples of blood were obtained from either the unused femoral vein or the external jugular veins for making determinations of the alkali reserve of the blood.

At the conclusion of the operative part of the experiments all of the animals were given intravenously 25 c. c. per kilogram of a warm 0.9 per cent. sodium chloride solution with the object in view of producing a free flow of urine.

### NORMAL DOGS.

#### *The Effect of a N/2 Solution of Hydrochloric Acid on Renal Function and Pathology.*

Eighteen normal dogs were used in this series of experiments. Six of the dogs were used for control experiments. These animals received the intravenous injection of isotonic sodium chloride solution but were not given the acid solution. The experiments lasted over a four hour period. For the first two hours of this period observations were made on carotid blood

---

\* Gréchant's Anesthetic. The animal is given 0.25 c. c. per kilogram of a 4 per cent. solution of morphine sulphate. This is followed in half an hour by 10 c. c. per kilogram of the following mixture: Chloroform 50 c. c.; alcohol and water, each 500 c. c.



pressure, urine flow and the reserve alkali of the blood at half hour intervals. For the last two hours of the experiments these observations were made at hour intervals.

The urine formed by the animals during the course of the experiments was collected at the end of the first two hour period and examined for albumin, casts and diacetic acid. At this stage of the experiment 1 c. c. of the usual solution of phenolsulphonephthalein was injected subcutaneously, the urine collected for the remaining two hour period, and the quantitative output of the dye determined.

At the end of the experiments the kidneys were removed and the tissue fixed in a corrosive acetic solution, Zenker's fluid, 95 per cent. alcohol, and in a 10 per cent. formaline solution. Sections from this tissue were stained with haematoxylin and eosin and with eosin and methylene blue. Fresh kidney tissue was frozen and sections made. Such sections were stained for lipid material by Herxheimer's Scharlach R method. The deductions which will be made concerning the pathology of the kidney in the various series of experiments are based on a histological study of such sections.

Immediately following the initial observations on urine flow and blood pressure, the animals of this series other than the animals of the six control experiments, were given intravenously 5 c. c. per kilogram of a N/2 solution of hydrochloric acid. At the end of the first hour of the experiments, the injection was repeated. The results obtained from such injections as contrasted with the control experiments will be found in Table 1, Study 1.

A study of this table shows that all of the animals prior to the commencement of the experiments were normal, in so far as renal function and a normal alkali reserve of the blood were concerned. The urine was free from albumin, glucose and diacetic acid. Tube casts were not present. The elimination of phenolsulphonephthalein by the respective animals in a two hour period varied from a minimum output of 58 to a maximum output of 84 per cent. The reserve alkali of the blood in the different animals varied from 8.0 to 8.1.

### *Control Experiments.*

Six normal dogs have been used for control experiments to ascertain the effect of Gréhan's anesthetic on renal function and the acid-base equilibrium of the blood during an experimental period of four hours and to note the changes in the kidney at the end of such a period of anesthesia. The results obtained in four of these animals, Experiments 1, 4, 7 and 10, are included in Table 1.

Immediately following the development of a satisfactory state of anesthesia the animals were given intravenously 25 c. c. per kilogram of 0.9 per cent. sodium chloride solution. Following the use of the solution of sodium chloride all of the animals developed a fair state of diuresis.





The flow of urine in the respective animals varied from 8 to 16 drops per minute. The systolic blood pressure in the different animals varied from a minimum of 112 mm. of mercury in the animal of Experiment 4, to a maximum of 138 mm. of mercury in the animal of Experiment 10.

At the end of the first hour period of the experiments the reserve alkali of the blood remained unchanged from the normal readings obtained prior to the use of the anesthetic. At this stage there had developed a slight reduction in the systolic blood pressure of all of the animals. The flow of urine in the animal of Experiment 10 showed an increase from 8 to 12 drops per minute. The urine flow in all of the other control animals had undergone a slight reduction. In the animal of Experiment 1, urine flow was reduced from 12 to 9 drops per minute, in Experiment 4, from 16 to 12 drops per minute, and in Experiment 7, from 10 to 7 drops per minute.

At the end of the second hour of the experiments but little change had taken place in the general condition of the animals or in the functional response of the kidneys.

The reserve alkali of the blood in all of the animals, except the animal of Experiment 7, had undergone a slight depletion. In the animal of Experiment 1, the reserve alkali was reduced from the normal reading of 8.1 to 8.0, in Experiment 4, from 8.0 to 7.95, and in Experiment 10, from 8.1 to 8.0.

All of the animals remained diuretic. The urine flow in the animal of Experiment 1 had increased from 10 to 16 drops per minute, and in Experiment 4 the animal showed an increase in urine formation from 8 to 17 drops per minute. In the remaining animals the flow of urine was reduced. In the animal of Experiment 7, the flow of urine was reduced from 10 to 8 drops and in the animal of Experiment 10, from 8 to 4 drops per minute.

At this stage of the experiments the systolic blood pressure of the respective animals showed but slight change from the normal readings. The lowest blood pressure was 106 mm. of mercury and the highest pressure was 127 mm. of mercury.

At the termination of the experiments, four hours after the initial observations, all of these control animals showed a reduction in urine formation. The flow of urine in the animal of Experiment 4 was reduced from 17 to 12 drops per minute, while in the animal of Experiment 10 urine formation had been reduced from 8 drops to 1 drop per minute.

The reserve alkali of the blood at the end of the four hour period not only failed to show a further depletion from that noted at the end of the second hour of the experiments, but in two of the animals there had developed during the last two hours of the period an attempt to restore the normal acid-base equilibrium of the blood. In the animal of Experiment 1, the reserve alkali of the blood had increased during the latter

half of the experimental period from 8.0 to 8.1. In the animal in Experiment 4, an increase had taken place from 7.95 to 8.0.

It is interesting to note that in these animals urine formation was in excess of the other control animals which during the period of anesthesia not only showed a reduction in the reserve alkali of the blood but also showed an inability to readjust the acid-base equilibrium of the blood.

During the period of anesthesia the systolic blood pressure in all of the animals showed some reduction. This was most marked in the animal of Experiment 10, in which a reduction occurred from the normal of 138 mm. of mercury to 110 mm. of mercury.

At the commencement of the last two hours of the experiments all of the animals were given subcutaneously 1 c. c. of a solution of phenolsulphonephthalein for a renal function test. The elimination of the dye was slightly reduced in all of the animals. The most marked reduction was in the animal of Experiment 10. The normal elimination for this animal before the experiment was 82 per cent. During the last two hours of the experimental period the output was 65 per cent. The eliminations of the dye by the other animals of the control series varied from a minimum output of 50 per cent. to a maximum output of 66 per cent.

Urine collected during the course of the control experiments with one exception was free from albumin and diacetic acid. The urine from the animal of Experiment 7 contained a trace of albumin but no casts.

The histological study of the kidneys from the control group of animals has shown but slight evidence of injury. The amount of stainable lipoid material is increased in the cells of the loops of Henle over that found in normal unanesthetized dogs. There occurs a moderate grade of cloudy swelling in the epithelium of some of the convoluted tubules. These cells have failed to show vacuolation or necrosis. Fig. 1, Study 1.

### *Conclusions Concerning the Control Group of Animals.*

1. Gréhan's anesthetic induces a satisfactory state of surgical anesthesia in normal dogs for a period of four hours.

2. During such a period of anesthesia there occurs in all of the animals some reduction in blood pressure. The maximum reduction has been 28 mm. of mercury.

3. All of the animals have remained diuretic during the course of the experiments but the flow of urine per minute has been reduced.

4. The elimination of phenolsulphonephthalein is reduced. The maximum output of the dye in a two hour period has been 66 per cent. and the minimum output 50 per cent.

5. With one exception the urine from all of the control animals has been free from albumin, casts and diacetic acid.

6. Three of the six control animals showed no reduction in the alkali reserve of the blood during the experimental period while the three remaining animals showed a depletion. Two of these animals during the last two hours of the experiments were able to re-establish a normal acid-base equilibrium of the blood to the reading obtained before the commencement of the experiments. Urine formation by these animals was in excess of the amount formed by those animals in which a re-establishment of the acid-base equilibrium of the blood was not effected. The animal of the series (Experiment 10) in which the most marked depletion in the alkali reserve of the blood developed was the least diuretic member of the group and the elimination of phenolsulphonethalein by this animal showed a greater reduction than was shown by any other member of the series of control animals.

The observation of outstanding interest in connection with this group of animals is that after the early stages of the experiments when a depletion in the alkali reserve of the blood has been induced, such normal animals may, as the duration of the experiments is prolonged, effect a re-establishment of this depletion. Associated with such a restoration in the blood chemical environment of the kidney urine formation and phenolsulphonethalein elimination is favored.

#### *The Effect of a N/2 Solution of Hydrochloric Acid on Renal Function and Pathology in Normal Dogs.*

Twelve animals were used in this series of experiments. The experiments were conducted in an identical manner with the previously described control group, with the exception that after the initial observations had been made the animals were given intravenously 5 c. c. per kilogram of a N/2 solution of hydrochloric acid. This injection was repeated at the end of the first hour of each experiment.

Eight representative experiments are included in Table 1, Study 1.

Studies made of the animals before the experiments showed them to be normal. The urine was free from albumin, glucose and diacetic acid. The elimination of phenolsulphonethalein by the respective animals varied from 58 to 84 per cent. The reserve alkali of the blood varied from 8.0 to 8.1.

Following the development of a state of anesthesia and the routine operative interference the dogs were given intravenously the usual injection of isotonic sodium chloride solution. A fair degree of diuresis ensued. The flow of urine by the different animals varied from 4 to 16 drops per minute.

The systemic blood pressure varied from 100 mm. to 135 mm. of mercury.

At this stage of the experiments the animals were given intravenously the solution of hydrochloric acid. Such injections induced a fast, followed by a slow and deep type of respiration which gradually became normal.

The immediate effect of such an injection was to reduce rapidly the reserve alkali of the blood in all of the animals. The maximum reduction from the normal of 8.0 to 7.75 developed in the animal of Experiment 16. In the animal of Experiment 12, the reserve alkali was reduced from 8.1 to 7.8, while in the remaining animals no reduction occurred below 7.9.

The result of such an injection in normal animals early in the experimental period is very constant. By the end of the second half hour period all of the animals had become markedly diuretic, and with one exception, they had either restored their normal acid-base equilibrium or they were in the process of such a restoration. For example: the animal of Experiment 16 had a normal alkali reserve of the blood of 8.0. Following the acid injection this was reduced to 7.75. The urine flow increased from 16 to 48 drops per minute and within an hour the reserve alkali had risen to 7.9. The animal of Experiment 13 had a normal alkali reserve of 8.0. Following the use of the acid solution the reserve alkali was reduced to 7.9. The flow of urine increased from 4 to 26 drops per minute and at the end of the first hour period the alkali reserve had been restored to the normal reading of 8.0.

During this early period of the experiments very slight changes developed in the systolic blood pressure of the animals. The blood pressure for the different animals varied from 100 to 140 mm. of mercury.

At the end of the second half hour period of the experiments the second and final injection of 5 c.c. per kilogram of a N/2 solution of hydrochloric acid was given. At this period of the experiments all of the animals were more freely diuretic than they were before the first acid solution was administered. The reserve alkali of the blood had either been restored to the normal or it had increased from the reduction induced by the first acid injection.

The second intravenous injection of the acid was followed by a disturbance in the breathing such as has been referred to and by an associated and abrupt reduction in the reserve alkali of the blood. The maximum reduction occurred in the animal of Experiment 16, in which the alkali reserve was reduced to the same reading as was obtained from the first acid injection 7.75. The least reduction occurred in the animal of Experiment 5, in which the reserve alkali was depleted from 8.05 to 7.9. The remaining animals showed an alkali reserve which varied from 7.8 to 7.85.



The reductions in the alkali reserve of the blood from the second injection of the acid solution were in general greater than from the first injection. The effect on urine formation and the rapidity with which an attempt is made to restore the acid-base equilibrium of the blood following the second injection of such a solution is strikingly different from that obtained when the first injection of an acid solution was used.

Following the second injection of the hydrochloric acid solution there was no increase in urine formation by any of the animals. With two exceptions urine formation was reduced, while in Experiments 12 and 16 no change occurred in the formation of urine. By the end of the first hour following the second dose of the hydrochloric acid solution a further reduction in urine formation had developed so that the maximum formation of urine by any animal of the series, Experiment 8, was 11 drops per minute, while the minimum output by the animal of Experiment 14, was 1 drop per minute.

The respective animals at this stage of the experiments manifest an inability to restore the acid-base equilibrium of the blood, and associated with this change in the physico-chemical state of the blood which is furnished the kidney there is a reduction in urine formation. For example, following the first injection of the acid solution in the animal of Experiment 16, the reserve alkali of the blood was reduced to 7.75, and within an hour it was restored to 7.9. The second injection of the acid solution in this animal reduced the alkali reserve to the same reading, 7.75, and at the end of an hour no change had taken place in the reading. No increase in urine formation occurred. In the animal of Experiment 13, the reserve alkali of the blood was reduced by the first acid solution from the normal of 8.0 to 7.9. At the end of an hour this reading had returned to the normal and the kidney was functionally active. Following the second injection of the acid solution the reserve alkali was reduced from 8.0 to 7.8, and at the expiration of an hour no change had taken place in the degree of depletion. Urine formation was decreased.

The response of these normal animals to a second injection of hydrochloric acid solution differed from the initial response of the animals in that in general a greater reduction in the alkali reserve of the blood develops; the animals do not become diuretic but on the contrary usually show a reduction in urine formation; and finally there is either no attempt on the part of the animals to restore the acid-base equilibrium of the blood in an hour period or the restoration is delayed as compared with the response of the animals to the first injection of such an acid solution.

During the remaining two hours of the experiments there occurred a gradual reduction in urine formation. At the end of the experiments the maximum output of urine by the animal of Experiment 18 was 8 drops per minute. The minimum output by the animal of Experiment 14, was 1 drop every two minutes.

During the last two hours of the experiments the reserve alkali of the blood showed a gradual increase but in none of the animals was the normal

reserve alkali re-established. These readings for the respective animals varied from 7.9 to 7.95.

The systolic blood pressure in the different animals has been well maintained throughout the experiments and has varied from 91 mm. of mercury to 125 mm. of mercury.

The effect of the acid solutions on renal function are clearly shown by the changes in the urine and by the elimination of phenolsulphonephthalein. The urine of all of the animals showed the presence of albumin. This varied from a faint trace to a heavy precipitate. Both hyalin and granular casts were present in the urine of eight of the twelve dogs. Diacetic acid was present in the urine of nine of the animals.

All of the animals showed a decided reduction in the elimination of phenolsulphonephthalein. The normal elimination of the dye by the animal of Experiment 5, was 84 per cent. As a result of the use of the acid solutions the elimination was reduced to 40 per cent. The animal of Experiment 13 had a normal elimination of 58 per cent. of the dye. Following the experiment this was reduced to 20 per cent.

The histology of the kidneys of these normal animals that have received two intravenous injections of N/2 hydrochloric acid shows the following changes. The amount of stainable lipoid material appearing in the loops of Henle is very greatly increased over that which could be demonstrated in the same location in the control group of animals. Furthermore, such stainable material appears as droplets in cells of the convoluted tubules. The epithelium of the tubules, and especially that of the convoluted tubules, shows advanced cloudy swelling and the accumulation of granules throughout the cytoplasm. The nuclei frequently stain imperfectly. The cells have but rarely shown vacuolation and necrosis of the epithelium has not been observed. Fig. 2, Study 1.

### *Conclusions Concerning the Group of Normal Animals That Received a N/2 Solution of Hydrochloric Acid.*

1. Following the intravenous administration of 25 c. c. per kilogram of an isotonic solution of sodium chloride to normal dogs, there has developed in eight of the twelve animals receiving such a solution a moderate grade of diuresis. The flow of urine in the different animals has varied from 4 to 16 drops per minute. In four normal animals that received such injections there was no increase in urine formation. The introduction of such an amount of fluid increases the volume of blood, induces a temporary hydremic state of the blood, and causes a transitory rise in blood pressure. With such conditions favorable for urine formation, the kidneys respond to this normal type of solution by either no increase in urine formation or by an increase which does not exceed the maximum output of 16 drops of urine per minute.



2. When these normal dogs, early in an experiment, before any damage has been induced in the kidneys, are given a solution of 5 c.c. per kilogram of a N/2 solution of hydrochloric acid intravenously, the solution being essentially not a normal solution, a change is induced in the physico-chemical state of the blood which at once throws into operation a mechanism in the kidney in an attempt to re-establish a normal blood chemical environment for the organism and for its own functional response. The use of such an acid solution at this early stage of the experiments caused a sudden reduction in the reserve alkali of the blood and a profuse diuresis. The greatest reduction in the reserve alkali of the blood was from a normal of 8.0 to 7.5. The most marked increase in urine formation occurred in the same animal. The flow of urine increased from 16 to 48 drops per minute.

Following the marked diuretic effect of such an acid solution in an uninjured kidney, the animals have within an hour either established a normal acid-base equilibrium of the blood or such an increase has occurred in the reserve alkali of the blood that the readings approach the normal.

There is a marked difference in the response of the kidney to these two types of solutions. The response of the normal kidney to an isotonic solution of sodium chloride, which does not deplete the reserve alkali of the blood, is either a negative response, or the diuretic effect is slight and not commensurate with the volume of fluid administered intravenously. The response of the normal kidney to an acid solution of less volume, but an essentially abnormal solution of such a composition that it induces a marked reduction in the alkali reserve of the blood, is a profuse diuresis, and associated with this functional response there occurs a partial or complete restoration of the acid-base equilibrium of the blood. As this equilibrium is attained there develops a decrease in urine formation.

3. A study of the urine formed by the different animals of the series at this stage of the experiments; viz., the end of the first hour, has shown the presence of albumin, and in the majority of the animals casts have also been present. It would appear that even though the normal kidney could respond to the changed physico-chemical state of the blood induced by the acid solution and in part re-establish a normal environment for the organism,

an injury was done to the functional unit, the kidney, which in large measure effected such a readjustment.

4. Following the second injection of the acid solution, the evidence of the renal injury induced by the first injection of such a solution is seen by the lack of response of the kidney to the second injection even though the reserve alkali of the blood in the various animals was depleted to a greater extent than was the case from the first injection. With an even greater demand for the restoration by the kidney of a departure from the normal in one of the fundamental physico-chemical states of the blood, this injured functional unit is unable to make the necessary response. The second injection of the acid solution increased the flow of urine in only one animal of the series. In the other animals the flow of urine was either reduced or it remained unaffected. During the remaining three hours of the experiments the reserve alkali of the blood was gradually increased but in none of the animals was the normal acid-base equilibrium of the blood attained.

*The Effect of Alkaline Solutions of Different Molecular Concentration on Renal Function and Pathology in Normal Dogs.*

Eighteen dogs were used in this series of experiments. The animals were subjected to an experimental technique identical with that outlined for the animals used in the first part of this study. The urine from all of these animals before the commencement of the experiments was normal. It did not contain albumin, casts, glucose, or diacetic acid. The elimination of phenolsulphonephthalein by the respective animals in a two hour period varied from a minimum output of 60 per cent. to a maximum output of 80 per cent. The reserve alkali of the blood was normal and varied from 8.0 to 8.1.

At the completion of the anesthesia with Gréhant's anesthetic the animals were given intravenously the usual preliminary injection of 25 c.c. per kilogram of 0.9 per cent. sodium chloride solution. Observations on urine flow, the reserve alkali of the blood, and systolic blood pressure were made at the usual intervals during the experiments. Eight of the animals were given intravenously 25 c.c. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride, while the remaining ten animals were given a similar amount per kilogram of a solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium chloride.

The following studies have been made on the ability of normal dogs to readjust the physico-chemical state of the blood, when this environment has departed from the normal by the introduction of alkaline solutions of

the concentrations above mentioned. Associated with such a study observations have been made on the functional and pathological response of the kidney when the blood chemical environment of this organ is made to depart from the normal by the use of such solutions. Twelve experiments representative of the results obtained in this group of animals are incorporated in Table 2, Study 1.

*The Effect of a Solution of Sodium Carbonate Equimolecular With 1.5 Per Cent. Sodium Chloride Solution on Renal Function and Pathology.*

A study of Experiments 7, 8, 10 and 12 of Table 2, which are representative of this group of animals, shows that following the intravenous injection of a solution of isotonic sodium chloride a fair degree of diuresis was established in the different animals. The flow of urine has varied from 6 to 12 drops per minute. The systolic blood pressure in the animals of the series varied from 105 to 126 mm. of mercury.

Following the intravenous injection of 25 c. c. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride, the reserve alkali of the blood in all of the animals was at once increased above the normal. In three of the animals the reserve alkali was increased from the normal of 8.0 to 8.2, in two animals from 8.1 to 8.2, while in the remaining animal the increase was from 8.0 to 8.15. Immediately following this degree of disturbance in the acid-base equilibrium of the blood there was established a free diuresis by all of the animals. The maximum formation of urine occurred in the animal of Experiment 7. Following the intravenous administration of an isotonic solution of sodium chloride to this animal the flow of urine was only 7 drops per minute. After the intravenous injection of an equal volume per kilogram of the sodium carbonate solution the flow of urine increased from 7 to 42 drops per minute. Associated with the free diuretic effect obtained from such a solution of sodium carbonate there occurred a rapid depletion in the alkali reserve of the blood, so that by the end of the first hour of the experiments the reserve alkali of the blood in all of the animals with two exceptions had returned to the normal. At this stage of the experiments there had occurred a moderate increase in the systolic blood pressure in all of the animals. The maximum rise in pressure of 23 mm. of mercury developed in the animal of Experiment 10.

At the end of the first hour of the experiments, while the animals were freely diuretic and in the process of establishing a normal acid-base equilibrium of the blood, a second injection of the sodium carbonate solution was given. The result obtained from the second administration of the alkali was to increase the alkali reserve of the blood again to a point beyond the normal. The increase did not exceed in any of the animals the readings obtained from the first injection.

The effect of a second injection of the carbonate solution on renal function was to induce a secondary increase in urine formation which was

[illegible]



not in excess of the amount of urine formed by the animals from the first injection of the alkaline solution.

Within half an hour following the second carbonate injection there occurred a reduction in the alkali reserve of the blood toward the normal in all of the animals except the animal of Experiment 10. In this animal the reserve alkali was not depleted but remained unchanged at a reading of 8.2.

During the remaining two hours of the experiments the animals continued to be freely diuretic, and the reserve alkali of the blood was gradually returned to the normal reading in all of the animals except those of Experiments 7 and 10. These animals had at the commencement of the experiments normal alkali reserve determinations of 8.0. At the end of the experiments the alkali reserve readings for these animals were 8.05. Such readings are within the limits of the normal.

Urine collected from all of the animals during the course of the experiments was free from albumin and casts. The urine from the animal of Experiment 8 contained diacetic acid.

Phenolsulphonephthalein determinations showed but slight reductions in the elimination of the dye when contrasted with its output by the animals before the commencement of the experiments. The maximum reduction in the elimination occurred in the animal of Experiment 10. The normal elimination for this animal was 80 per cent. At the termination of the experiment the output of the dye in a two hour period was 68 per cent.

The histological study of tissue obtained from the kidneys of these animals has in general shown no evidence of injury. Stainable lipid material does not appear in the convoluted tubule cells and can rarely be demonstrated in the cells of the loops of Henle. The epithelium lining the convoluted tubules appears shrunken and stains uniformly. The nuclei are hyperchromatic. Fig. 3, Study 1.

*Conclusions Concerning the Group of Normal Animals That Received a Solution of Sodium Carbonate Equimolecular With a 1.5 Per Cent. Solution of Sodium Chloride.*

1. Solutions of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride when given intravenously to normal dogs fail to induce such a degree of disturbance in the physico-chemical state of the blood as to render the kidney unable to readjust the altered physical state. The kidney responds to this degree of change in its blood chemical environment by establishing a free diuresis, and associated with this response the reserve alkali of the blood is reduced to the normal.

2. The degree of disturbance induced in the physico-chemical state of the blood by injections of such solutions fails to express itself in terms of an injury to the kidney. Urine formed during such periods is free from both albumin and casts and the elimination of phenolsulphonephthalein shows no more reduction than is induced by an anesthesia extending over a four hour period of experimentation.

*The Effect of a Solution of Sodium Carbonate Epuimolecular  
With a 3 Per Cent. Solution of Sodium Chloride on Renal  
Function and Pathology in Normal Dogs.*

The ten animals studied under the influence of this stronger solution of sodium carbonate were normal in so far as the functional response of the kidney was concerned. Urine from these animals was free from albumin and casts. The elimination of phenolsulphonephthalein by the respective animals varied from a maximum output of 75 per cent. to a minimum output of 58 per cent.

The reserve alkali of the blood has been normal. In the different animals the readings have varied from 8.0 to 8.1.

Before the use of the sodium carbonate solutions, the intravenous injection of an isotonic solution of sodium chloride induced a fair degree of diuresis in all of the animals. The formation of urine by the different animals varied from 6 to 15 drops per minute. Such injections caused a rise in systemic blood pressure which has varied in the different animals from 105 to 131 mm. of mercury.

Following the intravenous injections of the stronger solution of sodium carbonate, observations have been made during the four hour period of the experiments at the intervals previously indicated. The results obtained from these stronger alkaline solutions should be contrasted with the observations previously made when animals were subjected to the action of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride.

The immediate effect of injecting intravenously a solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium chloride is to cause an abrupt and marked rise in the alkali reserve of the blood. The maximum increase in the alkali reserve was from a normal reading of 8.1 to 8.4. The minimum increase was from a normal reading of 8.1 to 8.25.

Associated with this disturbance in the physico-chemical state of the blood is the fact that all of the animals have become freely diuretic. The degree of diuresis has varied in the different animals from 24 to 43 drops per minute. The most marked diuretic effect was obtained in the animals of Experiment 17, in which a urine flow of 6 drops per minute,



that was obtained from the use of 25 c. c. per kilogram of isotonic sodium chloride solution, was increased to 34 drops per minute from the use of a solution of sodium carbonate. The use of such solutions of sodium carbonate have induced a moderate rise in systolic blood pressure in all of the animals. The maximum rise of 12 mm. of mercury occurred in the animal of Experiment 14.

Associated with the profuse flow of urine provoked by such solutions there has occurred a rapid reduction in the reserve alkali of the blood in all of the animals. However, in only one of the animals was the normal acid-base equilibrium of the blood reestablished in the first hour period of the experiment. This result differs from the effect obtained when a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride was used. When the weaker solution was employed, all of the animals were able within the first hour of the experiment to reduce the reserve alkali of the blood either to the normal reading obtained for the animal before the commencement of the experiment, or to a reading which was within the limit of the normal.

At the end of the first hour of the experiment, with a freely diuretic state established in the animals, a second injection of the stronger solution of sodium carbonate was given. This injection resulted in a secondary increase in the alkali reserve of the blood which in all of the animals gave a higher reading than that obtained from the first injection. The maximum increase in the alkali reserve of 8.45 was obtained in the animal of Experiment 15.

Following this secondary disturbance in the acid-base equilibrium of the blood by a solution of sodium carbonate of the same volume and molecular concentration as the first solution, and with a rise in systemic blood pressure in all of the animals, there has developed but a slight increase in urine formation. The greatest increase of 17 drops per minute occurred in the animal of Experiment 22. In the other animals the increase in urine varied from 2 to 6 drops per minute.

From these observations it would appear that when the acid-base equilibrium of the blood was altered by the first injection of a solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium chloride, so that the kidney was unable to restore this equilibrium to the normal, the persistence of such an alteration in the physico-chemical state of the blood expressed itself locally in the kidney by rendering the kidney unable to react to a second injection of such a solution with the same degree of functional response as it was shown to possess at an earlier stage in the experiments.

During the remaining two hours of the experiments there occurred a very gradual decrease in the alkali reserve of the blood. Only three of the ten animals were able during this period to reestablish their normal acid-base equilibrium. The remaining seven animals of the series at the termination of the experiments had a reserve alkali of the blood which varied from 8.1 to 8.2.

Urine formation during this period progressively decreased, so that at the end of the experiments two of the animals were anuric, one animal was forming 1 drop of urine every two minutes, and two of the animals 1 drop per minute. The largest output of urine by any of the animals at this period was 10 drops per minute.

Changes similar in character to those just recorded as developing during the last two hours of the experiments, were observed in the previously discussed group of normal dogs that received a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride. However, in this former group of experiments none of the animals became anuric, the reduction in urine formation was not so marked, and all of the animals during the last two hours of the experiments were able to reestablish a normal acid-base equilibrium of the blood.

A study of the urine collected during the course of the experiments in which the animals received the stronger carbonate solutions shows that all of the animals developed an albuminuria and that casts occurred in the urine of four of the animals.

The elimination of phenolsulphonephthalein has been reduced in all of the experiments. The most marked reduction has occurred in the animal of Experiment 20, in which the elimination of the dye was reduced from the normal of 58 per cent. to 20 per cent. The urine from three of the animals contained diacetic acid.

The histological study of tissue from the kidneys of these animals which received the stronger solution of sodium carbonate shows in general a marked degree of cloudy swelling of the tubular epithelium. The epithelium of the convoluted tubules shows this change to an advanced degree, and in addition many of the cells are vacuolated and undergoing necrosis. Fig. 4, Study 1.

*Conclusions Concerning the Group of Normal Animals That Received a Solution of Sodium Carbonate Equimolecular With a 3 Per Cent. Solution of Sodium Chloride.*

1. A solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium chloride when given intravenously to normal dogs effects a disturbance in the animals which differs only in degree from that obtained when a weaker solution of sodium carbonate equimolecular with a 1.5 per cent solution of sodium chloride is employed. The stronger solutions induce a disturbance in the physico-chemical state of the blood of such a degree that the kidney is in general unable to readjust this altered physico-chemical state and establish a normal blood chemical environment for the organism.

2. The inability of the organism to effect such a readjustment is shown locally by the development of a kidney injury which expresses itself functionally in the appearance of albumin and casts in the urine, a decrease in the elimination of phenolsulphonephthalein, and by a decrease in urine formation or the establishment of an anuria.

3. These experiments in which solutions of different molecular concentrations of sodium carbonate have been employed indicate that the normal organism, very largely through the activity of the kidneys, is able to readjust to the normal a certain degree of disturbance in the acid-base equilibrium of the blood without the kidney becoming injured during such an adjustment. When, however, the physico-chemical state of the blood undergoes too great a departure from the normal from the use of such an alkaline solution, the environment of the kidney becomes so changed that its functional response is interfered with and a normal acid-base equilibrium of the blood is not established.

4. In normal dogs in which there has developed from the use of an alkaline solution or a  $N/2$  solution of hydrochloric acid an inability to effect a readjustment in the acid-base equilibrium of the blood, the type of renal injury is in general the same. This injury is primarily one of cloudy swelling of the renal epithelium which is most marked in the cells of the convoluted tubules. The more advanced changes of degeneration consist in edema, vacuolation and more rarely an early necrosis of the tubular epithelium.

## REFERENCES.

## STUDY I.

1. Henderson, L. J. *Jour. Biol. Chem.*, 9, 1911, 403.
2. Lassar, O. *Arch. f. d. ges. Physiol.*, 9, 1874, 44.
3. Walter, F. *Archiv. f. exp. Path. u. Pharmakol.*, 7, 1877, 148.
4. Loeb, J. *Pflüger's Archiv.*, 71, 1898, 457.
5. Loeb, J. *Arch. f. d. ges. Physiol.*, 101, 1904, 340.
6. Loeb, J. *Arch. f. d. ges. Physiol.*, 103, 1904, 503.
7. Hoppe-Seyler, F., and Araki, T. *Ztschr. f. physiol. Chem.*, 15, 1891, 335 and 546.
8. Hofmeister, F. *Archiv. f. exp. Path. u. Pharmakol.*, 27, 1890, 210.
9. Fischer, Martin H. *Oedema. New York*, 1910.
10. Fischer, Martin H. *Nephritis. New York*, 1912.
11. Henderson, L. J., Palmer, W. W., and Newburgh, L. H. *J. Pharmacol. and Exp. Therapeutics*, 5, 1913, 449.
12. Bornstein, A., and Lippmann, A. *Ztschr. f. klin. Med.*, 86, 1918, 345.
13. Fitz, R., Alsberg, C. L., and Henderson, L. J. *Amer. J. Physiol.*, 18, 1907, 113.
14. Nagayama, T. *Amer. J. Physiol.*, 51, 1920, 434.
15. Jewell, Minna E. *J. Exp. Zoology*, 30, 1920, 461.
16. MacNider, Wm. deB. *J. Exp. Med.*, 26, 1917, 1.
17. MacNider, Wm. deB. *Science, N. S.*, 66, 1917, 643.
18. MacArthur, J. W. *Amer. J. Physiol.*, 54, 1920, 138.



## DESCRIPTION OF FIGURES.

## STUDY I.

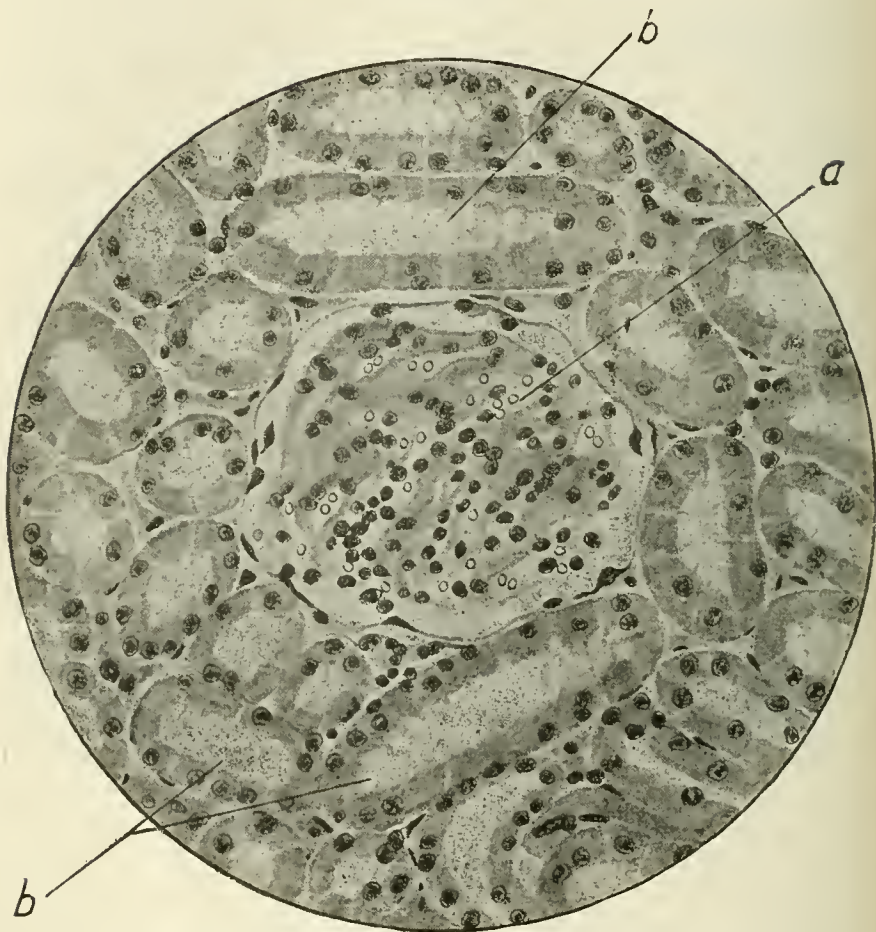


Fig. 1. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the kidney of the normal control animal of Experiment 4. Study 1. Table 1.

The animal was anesthetized by Gréhan's anesthetic for a period of four hours. With the completion of a satisfactory state of surgical anesthesia the animal was given 25 c. c. per kilogram of a 0.9 per cent. solution of sodium chloride. The animal remained freely diuretic throughout the experiment. The flow of urine at the end of the experiment was 11 drops per minute. The elimination of phenolsulphonephthalein was only reduced from the normal of 65 per cent. to 50 per cent. No albumin or casts appeared in the urine. The reserve alkali of the blood was normal at the termination of the experiment. At A, is shown a normal glomerulus. At B, are shown convoluted tubules in which the epithelium appears granular but shows only slight swelling. The nuclei of the cells stain well. The epithelium shows no evidence of vacuolation or necrosis.

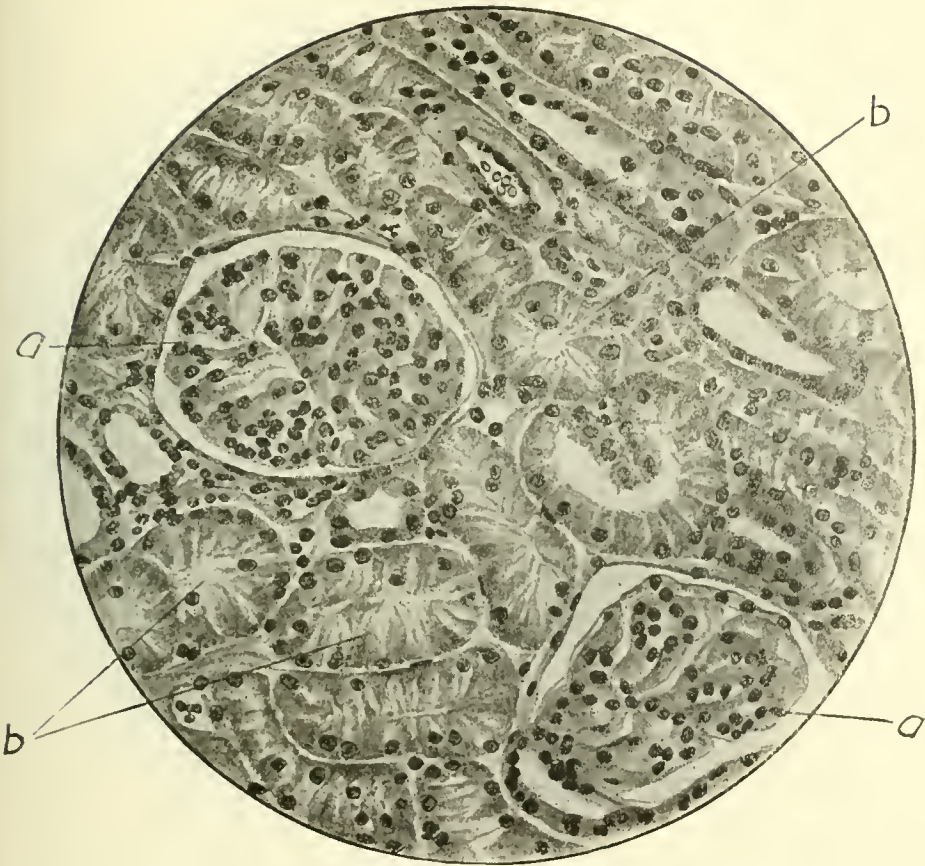


Fig. 2. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the kidney of the animal of Experiment 14, Study I, Table 1. The animal received two injections of a solution of N/2 hydrochloric acid. The animal was unable to reestablish and maintain a normal acid-base equilibrium of the blood following such injections. The reserve alkali of the blood at the termination of the experiment was 7.95. Albumin and casts appeared in the urine. The elimination of phenolsulphonephthalein was reduced from the normal output of 60 per cent. to 35 per cent. At the termination of the experiment the animal was forming only 1 drop of urine every two minutes. At A, are shown normal glomeruli. At B, are shown convoluted tubules with edematous and vacuolated epithelium. In some of the tubules the edema is so severe as to occlude the lumen of the tubules. The nuclei of the cells vary in the intensity of their staining power.



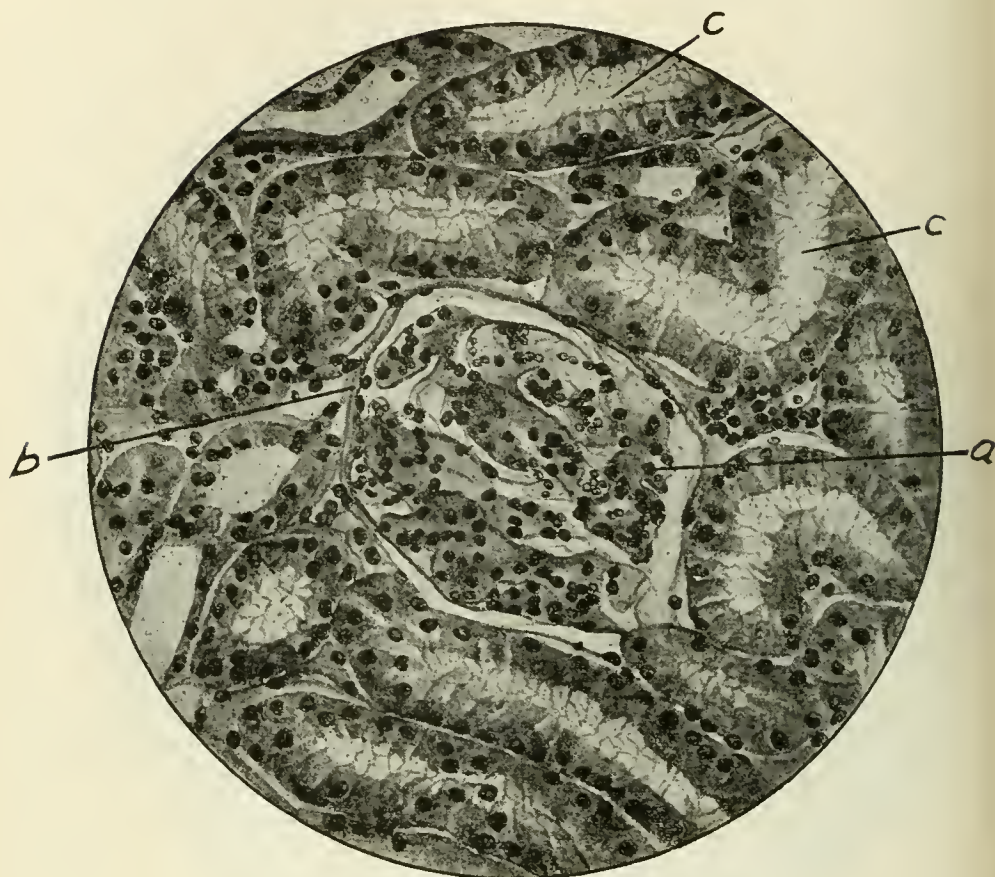


Fig. 3. Camera lucida drawing. Leitz Oc. 2. obi. 6.

The figure is from the kidney of the animal of Experiment 8, Study 1. Table 2.

The animal received two intravenous injections of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride. The animal was able to establish and maintain the normal reserve alkali of the blood of 8.1. No albumin or casts appeared in the urine. The elimination of phenolsulphonephthalein during the experiment was only reduced from the normal output of 63 per cent. to 55 per cent. At the termination of the experiment the animal was forming 12 drops of urine per minute.

At A, is shown a normal glomerulus, and at B, the capsule of the glomerulus. At C, are shown convoluted tubules in a good state of preservation. The epithelium of such tubules is not swollen. The lumen of the tubules are prominent. The nuclei of the cells stain intensely.

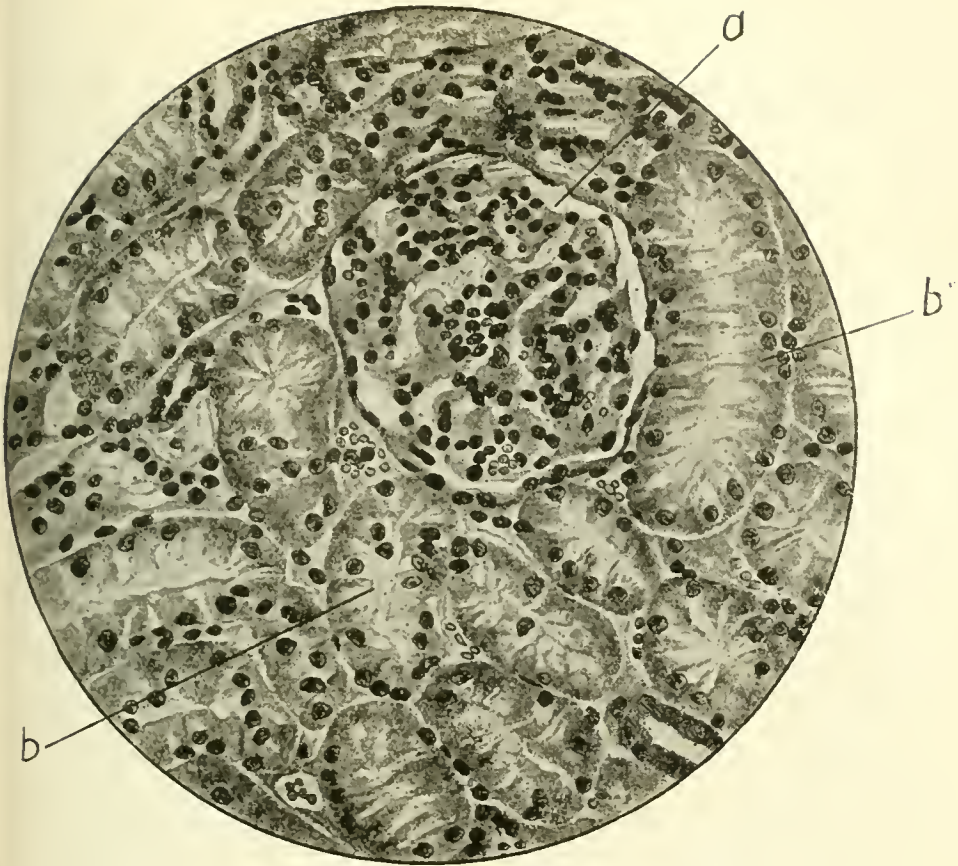


Fig. 4. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the kidney of the animal of Experiment 15, Study 1. Table 2.

The animal received two intravenous injections of a solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium chloride. The animal was unable to establish and maintain a normal acid-base equilibrium of the blood. Albumin and casts appeared in the urine. The elimination of phenolsulphonephthalein was reduced from the normal of 66 per cent. to 24 per cent. At the termination of the experiment the animal was anuric.

At A, is shown a normal glomerulus. At B, are shown convoluted tubules with the epithelium edematous and vacuolated. The lumina of many of the tubules are closed by the edematous cells. The nuclei of the cells vary in their staining power.



STUDIES CONCERNING THE INFLUENCE OF A  
DISTURBANCE IN THE ACID-BASE EQUILIBRIUM  
OF THE BLOOD ON RENAL FUNCTION  
AND PATHOLOGY.\*

STUDY II.

THE EFFECT OF ACID AND ALKALINE SOLUTIONS ON RENAL  
FUNCTION AND PATHOLOGY IN NATURALLY  
NEPHROPATHIC DOGS.

Wm. deB. MacNider

*The Laboratory of Pharmacology, the University of North Carolina.*

In the first study of this series an investigation was made of the stability of the acid-base equilibrium of the blood in normal dogs when anesthetized by Gréhant's anesthetic over a period of four hours and of the ability of such animals to readjust this fundamental reaction of the blood when it was disturbed by the intravenous administration of an acid or an alkaline solution. The pathological effect of such solutions was observed by a study of renal function and the histological changes induced in the kidney.

In the following study similar observations will be made on the disturbance induced in naturally nephropathic animals by the introduction of such solutions, of the ability of a previously damaged kidney to readjust a disturbance in the acid-base equilibrium of the blood, and of the changes which such solutions induce in the functional and pathological response of the kidney.

In recent years numerous investigations have appeared which have had as their object a study of the acid-base equilibrium of the blood in the various types of nephropathies and of the relationship which such a disturbance has to certain changes in renal function. These studies have been very largely concerned with the development of the acid intoxication which occurs in such renal injuries and with a discussion as to whether such a depletion in the alkali reserve of the blood should be considered as the cause for certain renal injuries, or whether the disturbance should be interpreted as a retention intoxication. The investigation which is to follow is not primarily concerned with these ques-

---

\*Aided by a grant from the Rockefeller Institute for Medical Research.



tions, but is concerned with the relative ability of the naturally nephropathic kidney as contrasted with the normal kidney to readjust such a fundamental disturbance in the blood chemical environment of the organism. The study, furthermore, takes into consideration the severity of the pathological changes developing in the kidney of such animals and the degree to which the functional response of the kidney is interfered with.

The earlier literature dealing with the question of the development of an acid intoxication in various types of nephropathic processes has been reviewed by Ewing<sup>1</sup>, and the more recent literature by Sellards.<sup>2</sup> In the present paper references will be made only to more recent papers which deal with the general question of the disturbance in the acid-base equilibrium of the blood in nephropathic animals.

In 1909 Von Hösslin<sup>3</sup> observed a rather definite relationship between the acidity of the urine and the amount of albumin and casts in the urine. Later than this Fischer<sup>4</sup>, as a result of his work on edema, applied his findings to certain changes developing in the kidney in various forms of nephritis and emphasized the importance of the acidosis occurring in such states. In 1913 Palmer<sup>5</sup> and Henderson, following a series of clinical studies on the acid-base equilibrium of the blood and the nature of acidosis, decided that a condition of acidosis existed in a great variety of pathological conditions. They furthermore made suggestions concerning the rational use of alkalis as therapeutic agents. At a later date the same investigators<sup>6</sup> demonstrated in a series of experiments the constant occurrence of an acidosis in one type of nephritis and of its frequent occurrence in other types. In these studies they were able to demonstrate a retention of alkali following the use of sodium bicarbonate. In 1915 Peabody<sup>7</sup> made a study of the acidosis of chronic nephritis in connection with functional renal tests and came to the conclusion that the acidosis in such conditions was a retention phenomenon. Very recently Chase and Myers<sup>8</sup> in a study of the acidosis of nephritis have come to the conclusion that all fatal cases of nephritis with marked nitrogen retention have a severe acidosis, sufficient in many instances to be the cause of death.

In recent years several investigations have been made in this laboratory concerning the stability of the acid-base equilibrium of the blood in animals of different age periods<sup>9</sup>, and in naturally nephropathic animals. These observations have been extended to a study of the susceptibility of such animals to the toxic effect of the general anesthetics<sup>10, 11</sup> and the degree of disturbance induced in the acid-base equilibrium of the blood by the use of such anesthetic substances. Other studies have dealt with the restoration of the acid-base equilibrium of the blood in animals recovering from an acute nephropathic process<sup>12, 13</sup> and of the disturbance which is induced in this equilibrium when an acute injury is superimposed on a chronic renal injury.<sup>14</sup>

In 1903 Pearce,<sup>15</sup> in an experimental study of nephrotoxins, used in his investigation the sera from dogs with a spontaneous nephritis. Later, in 1908, Ophüls<sup>16</sup> noticed that chronic nephritis in dogs is a common disease. Dayton,<sup>17</sup> in an investigation of the frequency of such a condition in dogs, found in a study of twenty-one animals only one dog with what he considered normal kidneys. In 1916 an extensive study was made in this laboratory of the naturally acquired nephropathy of the dog and of the physiological response of such kidneys to diuretic substances.<sup>18, 19</sup> In these studies the frequency of the occurrence of a chronic nephropathy in the dog was confirmed and the various nephropathic processes were classified for purposes of study. The classification showed very clearly that with few exceptions the chronic kidney injury in the dog is primarily a glomerulonephropathy and that extensive changes may develop in the glomeruli before the tubules become implicated in the pathological process. The animals employed in the following study have had this type of chronic naturally-acquired kidney injury.

*The Effect of a N/2 Solution of Hydrochloric Acid on Renal Function and Pathology in Naturally Nephropathic Dogs.*

Fifteen naturally nephropathic animals were used in this series of experiments. The preliminary observations and the experimental procedures are identical with those outlined in Study 1, in which normal dogs were employed. Four of these animals were used for control experiments. They were anesthetized by Gréhant's anesthetic and given intravenously 25 c. c. per kilogram of a 0.9 per cent. solution of sodium chloride. The remaining animals were anesthetized and given a similar solution of sodium chloride and at two periods of the experiments were given intravenously 5 c. c. per kilogram of a N/2 solution of hydrochloric acid. The observations on all of the control animals and on eight of the animals subjected to the action of a solution of hydrochloric acid have been included in Table 1 of the present study.

The observations made on these animals during the period of preliminary study show them to be naturally nephropathic. The urine from all of the animals contained albumin and with one exception tube casts. The appearance of the albumin was variable. In three of the fifteen animals its presence was not constant, but varied from day to day.

The elimination of phenolsulphonephthalein in a two hour period was uniformly below the normal and varied from a minimum output of 33 per cent. to a maximum output of 58 per cent. The reserve alkali of the blood in these naturally nephropathic animals in which the chronic pathology is largely localized in the glomeruli was normal with two exceptions. In these two animals, Experiments 7 and 12, the reserve alkali was 7.95. In such animals, with a reduction in the reserve alkali of the blood, the elimination of phenolsulphonephthalein was low, and the amount of albumin in the urine or the number of casts was more marked than in any of the other animals.



The animal of Experiment 7 had a reserve alkali of 7.95, an elimination of phenolsulphonophthalein of 48 per cent.; and while only a trace of albumin was present in the urine, there were very numerous hyaline and finely granular casts. The animal of Experiment 12 had a reserve alkali of 7.95, an elimination of phenolsulphonophthalein of 33 per cent., and a heavy trace of albumin with casts.

### *Control Experiments With Naturally Nephropathic Animals.*

These animals were not subjected to the action of a solution of hydrochloric acid. Following the establishment of a state of anesthesia from Gréhant's anesthetic and after the administration of the usual solution of isotonic sodium chloride, a fair degree of diuresis was obtained in the various animals. The flow of urine varied from 10 to 21 drops per minute. The systolic blood pressure in the respective animals has varied from 100 mm. to 134 mm. of mercury.

At this early period of the experiments the reserve alkali of the blood remained unchanged except in the animal of Experiment 2, in which the reserve alkali was reduced from the normal reading of 8.05 to 8.0.

By the end of the second half hour period of the experiments very little change had taken place in the rate of urine formation or in the systolic blood pressure of the different animals. The reserve alkali of the blood at this period was reduced in all of the animals except one. The maximum reduction was obtained in the animal of Experiment 1, in which the reserve alkali was reduced from the normal of 8.0 to 7.9.

At the termination of the third half hour period of the experiments, urine formation had undergone a marked reduction. The animal of Experiment 2 was anuric. Such changes in urine formation have not been associated with any marked fall in systolic blood pressure. In the anuric animal of Experiment 1, systolic blood pressure at this period of the experiment was 128 mm. of mercury, 4 mm. in excess of the normal blood pressure reading for the animal.

In the animal of Experiment 5, urine formation had been reduced from the normal flow of 18 drops per minute to 11 drops per minute. The normal blood pressure for this animal was 134 mm. of mercury. Associated with the reduction in urine formation the blood pressure had fallen only to 130 mm. of mercury.

A study of the acid-base equilibrium of the blood at this period of the experiments, when urine formation is either being reduced or the animals rendered anuric, shows a marked depletion in the alkali reserve in all of the animals. The reserve alkali of the blood in the animal of Experiment 5, in which urine formation was reduced from 18 to 11 drops per minute, was reduced from 8.1 to 7.9. In the anuric animal of Experiment 2, the reserve alkali had undergone a depletion from 8.0 to 7.85.

Throughout the remainder of the experiments which lasted for four hours, there was a progressive decrease in urine formation by all of the

animals. At the termination of the experiments the maximum urine formation by the animal of Experiment 5 was 4 drops per minute. The animals of Experiments 1 and 4 were forming 2 drops of urine per minute, while the animal of Experiment 2, that became anuric early in the experiment, remained anuric. The systolic blood pressure in these animals did not undergo any marked reduction. The blood pressure for the different animals varied from 98 to 120 mm. of mercury.

The reserve alkali of the blood, although depleted from the normal in all of the animals, remained very constant, with one exception, after the initial depletion which occurred at the end of the third half hour period of the experiments. The readings for all of the animals, except the anuric animal of Experiment 2, were 7.9. In this latter animal the reserve alkali of the blood at the termination of the experiment was reduced to 7.8.

A study of the urine formed by the different animals during the course of the experiments showed an increase in the amount of albumin over that normally present in the urine. Diacetic acid appeared in the urine of all of the animals examined for this substance.

The elimination of phenolsulphonephthalein was reduced in all of the animals during the period of anesthesia. In the animal of Experiment 4, the elimination of the dye was only reduced to 40 per cent. from the normal output of 50 per cent. In the animal of Experiment 2, that became anuric during the period of anesthesia, the elimination of the dye was reduced from 45 per cent. to a trifle less than 10 per cent.

A study of the histological changes induced in the naturally nephropathic kidney by an anesthesia of four hours duration from Gréhan's anesthetic shows that the vascular tissue fails to develop an acute injury. The glomerular capillaries were well filled with blood when the chronic fibrous changes and hyalinization did not exclude the circulation. No exudate or actual hemorrhage was observed in the subcapsular spaces. The chronic pathology of the glomeruli consisted of those capsular and intracapillary changes common to an early or moderately advanced glomerulonephropathy.

The toxicity of the anesthetic for the kidney is expressed by changes in the tubular epithelium. The amount of stainable lipoid material is increased in the cells of the loops of Henle and in the convoluted tubule epithelium. The epithelium of the tubules in general shows cloudy swelling and a moderate grade of vacuolation. In the anuric animal of Experiment 2, the epithelium of the convoluted tubules showed an advanced grade of swelling, vacuolation, and in many of the cells a well advanced necrosis. Fig. 1. Study II.

### *Conclusions Concerning the Control Group of Naturally Nephropathic Animals.*

1. Following an anesthesia of four hours duration from Gréhan's anesthetic in naturally nephropathic dogs, there is more

evidence of its toxic effect than develops from a similar period of anesthesia in normal dogs.

2. This increased toxic effect is shown by the naturally nephropathic animals by the anesthetic inducing an earlier and more marked disturbance in the acid-base equilibrium of the blood which is associated with a reduction in urine formation and which is not accompanied by a reduction in systolic blood pressure.

3. In normal dogs, following the initial disturbance in the acid-base equilibrium of the blood induced by the anesthetic, there occurred a readjustment of this equilibrium which was associated with an increase in urine formation.

In naturally nephropathic animals after the anesthetic has induced a disturbance in the acid-base equilibrium of the blood, there is no attempt on the part of the animals to re-establish this balance. The degree of depletion in the alkali reserve of the blood remains unchanged, or it undergoes a further reduction as the anesthesia progresses.

4. In naturally nephropathic animals in which the blood has undergone such a physico-chemical change that the kidney is no longer furnished a normal environment in which to functionate, there occurs a reduction in urine formation or the establishment of an anuria. The elimination of phenolsulphonephthalein is reduced and an increased amount of albumin appears in the urine. This altered environment of the kidney is shown histologically by the development of acute changes of a degenerative character in the tubular epithelium and by the lack of such changes in the glomeruli which are the seat of the primary chronic injury.

*The Effect of a N/2 Solution of Hydrochloric Acid on Renal Function and Pathology in Naturally Nephropathic Dogs.*

Eleven of the naturally nephropathic animals of this series were subjected to the same experimental technique as has been outlined for the control group of animals, and in addition at two periods of the experiments were given intravenously 5 c. c. per kilogram of a N/2 solution of hydrochloric acid. The results obtained in eight of these animals are included in Table 1, Study II.

At the completion of the anesthesia from Gréhant's anesthetic and following the intravenous injection of 25 c. c. per kilogram of a 0.9 per cent. solution of sodium chloride, these animals showed a fair degree of

Sample No.	Station	Depth (ft.)	Soil Type	Moisture (%)	Temperature (°C)	Specific Gravity	Unit Weight (pcf)	Void Ratio	Porosity (%)	Shrinkage (%)	Swelling (%)	Consistency	Notes
1	Top of alluvium	0.0	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
2	Top of alluvium	0.5	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
3	Top of alluvium	1.0	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
4	Top of alluvium	1.5	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
5	Top of alluvium	2.0	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
6	Top of alluvium	2.5	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
7	Top of alluvium	3.0	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
8	Top of alluvium	3.5	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
9	Top of alluvium	4.0	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
10	Top of alluvium	4.5	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
11	Top of alluvium	5.0	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
12	Top of alluvium	5.5	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis
13	Top of alluvium	6.0	Clay	15.0	20.0	2.65	120.0	0.65	65.0	0.0	0.0	Stiff	Sample for analysis



diuresis. The flow of urine varied in the different animals from an output of 4 to 18 drops per minute. The systemic blood pressure in the respective animals varied from a minimum pressure of 100 mm. of mercury to a maximum pressure of 140 mm. of mercury.

At this stage of the experiments the animals were given the first intravenous injection of a solution of hydrochloric acid. The effect from such an injection was to induce a marked disturbance in the acid-base equilibrium of the blood in all of the animals. The degree of depletion in the alkali reserve varied in the different animals. In the animal of Experiment 8, the reserve alkali was reduced from 8.1 to 8.0; in the animal of Experiment 10, from the normal of 8.0 to 7.8. Associated with such changes in the alkali reserve of the blood in naturally nephropathic animals, there occurred a reduction in urine formation in all of the animals with two exceptions. In two of the animals the output of urine increased 2 drops per minute. This observation is in striking contrast with the results obtained when a similar solution of hydrochloric acid was administered to normal dogs. In such animals a profuse flow of urine followed the acid injection. (Study I.)

In the naturally nephropathic animals no marked change developed in the systolic blood pressure from the first injection of the acid solution. The blood pressure readings for the respective animals varied from a minimum reading of 105 mm. of mercury in the animal of Experiment 6 to the maximum reading of 142 mm. of mercury in the animal of Experiment 4.

At the end of the second half-hour period of the experiments the reserve alkali of the blood remained reduced below the normal in all of the animals. Four of the animals, as the experiments had progressed during this period, showed a progressive reduction in the reserve alkali of the blood, which was most marked in the animal of Experiment 12, in which the reserve alkali determination now gave a reading of 7.7. In the remaining animals the reserve alkali remained unchanged. In none of the animals was there any evidence of an attempt at a restoration of the acid-base equilibrium of the blood. At this period of the experiments, the end of the second half-hour, two of the animals had become anuric, and with the exception of the animal of Experiment 10, in which the formation of urine remained at 2 drops per minute, urine formation by all of the remaining animals had been greatly reduced.

The reduction in urine formation shows no correlation with a fall in systemic blood pressure. In the anuric animal of Experiment 12, the blood pressure at this period was 130 mm. of mercury as opposed to a pressure of 128 mm. of mercury when the animal was forming 4 drops of urine per minute.

Following these observations, at the end of the first hour of the experiments, the animals were given the second and final injection of 5 c. c. per kilogram of a N/2 solution of hydrochloric acid. The injection of the acid induced a secondary and more marked disturbance in the acid-base equi-

librium of the blood than was obtained from the first injection. The maximum reduction of the alkali reserve of the blood to 7.6 occurred in the animals of Experiments 12 and 15. The minimum reduction to 7.9 developed in the animal of Experiment 11. Following this degree of change in the physico-chemical state of the blood, urine formation which had previously been reduced, remained unchanged in three of the animals, Experiments 8, 10 and 15; two of the animals continued anuric, while in the remaining animals there was a further reduction in urine formation.

The blood pressure determinations in the respective animals varied from a minimum pressure of 100 mm. of mercury in the animal of Experiment 6, to a maximum blood pressure of 135 mm. of mercury in the animal of Experiment 14.

From this stage of the experiments until their termination at the end of a four hour period, the disturbance induced in the acid-base equilibrium of the blood showed either a progressive increase of the degree of disturbance or remained unchanged from that induced by the second injection of the hydrochloric acid solution. At the end of the experiments the lowest reserve alkali readings which occurred in four of the animals, Experiments 6, 7, 12 and 15, were 7.6. The highest reading of 7.8 was obtained in the animal of Experiment 11. The reserve alkali determinations for the remaining animals was found between these two extremes. There was no attempt on the part of any of the animals to re-establish a normal acid-base equilibrium of the blood.

Associated with this degree of departure from the normal in the acid-base equilibrium of the blood and with an inability to attempt a re-establishment of the equilibrium, all of the animals after the first hour of the experiments showed a marked reduction in urine formation, so that by the end of the third hour of the experiments six of the eleven animals were anuric, and by the termination of the experiments, one hour later, all of the animals, with one exception, were anuric. This animal, Experiment 8, was forming 1 drop of urine per minute.

This reduction in urine formation cannot be ascribed to an excessively low systolic blood pressure in any of the animals. At the conclusion of the experiments the systolic blood pressure in the different animals varied from a minimum of 96 mm. to a maximum of 110 mm. of mercury.

Urine collected from the animals during the course of the experiments showed a heavy precipitate of albumin and the presence of numerous hyalin and finely granular casts. The amount of albumin was in excess of that found in the urine prior to the experiments. Diacetic acid was present in the urine of all of the animals examined for this substance. In those animals in which urine formation was sufficient to permit a phenolsulphonephthalein determination, the elimination of the dye in a two hour period varied from a mere trace to a maximum output of 18 per cent.

The histological study of the kidneys of the naturally nephropathic animals anesthetized by Gréhant's anesthetic and subjected to a disturbance in the acid-base equilibrium of the blood from two injections of



5 c. c. per kilogram of a N/2 solution of hydrochloric acid showed the following changes.

The changes are similar to those described for the control group of naturally nephropathic animals which were anesthetized by Gréhant's anesthetic and which were not given the acid injections, except that the use of the acid solution in this latter group of animals very greatly increased the severity of these changes.

The glomeruli showed no evidence of an acute injury. The chronic glomerular pathology consisted of the same type of chronic changes previously described.

The tubular epithelium shows a marked increase in stainable lipid material over that which could be demonstrated in the control group of animals.

The tubular epithelium in general has shown advanced cloudy swelling, vacuolation and an early necrosis. These changes are least marked in the epithelium of the collecting tubules and most marked in the convoluted tubule epithelium. In the latter location the epithelium is frequently so edematous as to obliterate the lumen of the tubules.

The nuclei show fragmentation and fail to stain and in such cells necrosis may be seen in an advanced stage. Fig. 2. Study II.

*Conclusions Concerning the Effect of Injections of a N/2 Solution of Hydrochloric Acid on Renal Function and Pathology in Naturally Nephropathic Dogs.*

1. Naturally nephropathic animals show an early and marked susceptibility to the toxic effect of intravenous injections of N/2 solutions of hydrochloric acid. The toxic effect is first shown by a severe disturbance in the acid-base equilibrium of the blood, which is soon followed by a reduction in urine formation.

2. The response of naturally nephropathic animals to such solutions differs in several particulars from the response of normal animals. Study 1. In the first place the degree of disturbance in the acid-base equilibrium of the blood is more marked in naturally nephropathic animals than in normal animals, even though the naturally nephropathic animal may have been able to maintain prior to the experiment a normal acid-base equilibrium of the blood. In the second place, when normal animals are given such solutions of hydrochloric acid, there occurs very rapidly an attempt to re-establish the normal physico-chemical state of the blood. The increased hydrogen ion content of the blood induces

a free diuresis, and, associated with the rapid output of urine, the acid-base equilibrium of the blood is either restored to the normal or an attempt is made on the part of the organism in this direction.

When such acid solutions are administered to naturally nephropathic animals with a normal acid-base equilibrium of the blood, or with a blood slightly depleted in its alkali reserve, the disturbance induced in the acid-base equilibrium is so far reaching in its effect, the environment of the kidneys in terms of the physico-chemical state of their blood supply is so changed, that either no diuretic effect is obtained or the flow of urine is but slightly increased for a short period.

It would appear that in naturally nephropathic animals, such as have been used in this study, some mechanism in the kidney is normally under the strain of in part maintaining a normal acid-base equilibrium of the blood for the organism as a whole, and for the functional response of the kidney in particular. When this mechanism is subjected to the additional strain induced by a period of anesthesia, plus the introduction of an acid solution, it becomes ineffective, and this lack in its effectiveness is shown by a more marked reduction in the alkali reserve of the blood than occurs in normal animals. There is an inability on the part of the animal to readjust the physico-chemical disturbance in the blood in so far as a restoration of the acid-base equilibrium of the blood is concerned.

3. A further study of the course of the experiments in naturally nephropathic animals shows that after the acid-base equilibrium of the blood has once undergone the degree of disturbance induced by the first injection of an acid solution, and the blood chemical environment of the kidney has become so changed that the renal function in this environment is either reduced or arrested, the animals are throughout the experiments unable to readjust a balance between the hydrogen and hydroxyl ion content of the blood. As the experiments progress, the disturbance in the acid-base equilibrium of the blood increases, and associated with this further alteration in the physico-chemical state of the blood the kidneys cease to act as functional units. With two exceptions, all of the animals of the series were anuric at the termination of the experiments.

4. The greater toxicity of an acid solution for the naturally nephropathic kidney than for the normal kidney is furthermore shown anatomically by the more extensive degenerative changes in the tubular epithelium and functionally by the relative inability of such animals to eliminate phenolsulphonephthalein.

*The Effect of Alkaline Solutions of Different Molecular Concentration on Renal Function and Pathology in Naturally Nephropathic Dogs.*

Eighteen naturally nephropathic animals were used in this series of experiments. Eight of these animals were studied following the intravenous injection of 25 c. c. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride. The remaining animals of the series received 25 c. c. per kilogram of a solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium carbonate. The effect of these solutions was observed over the usual experimental period of four hours. The results obtained in twelve representative experiments are included in Table 2. Study II.

*The Effect of a Solution of Sodium Carbonate Equimolecular With a 1.5 Per Cent. Solution of Sodium Chloride on Renal Function and Pathology.*

Of the eight animals studied under the influence of a solution of sodium carbonate of the above-mentioned strength, all were found to be naturally nephropathic. The urine contained albumin and casts. The elimination of phenolsulphonephthalein in a two hour period varied in the respective animals from 38 to 50 per cent. The reserve alkali of the blood in the animal of Experiment 5 was 7.95. In the remaining animals these readings were within the normal and varied from 8.0 to 8.5.

Following the development of a state of anesthesia, the animals were given the usual preliminary injection of 25 c. c. per kilogram of isotonic sodium chloride solution. There developed a fair degree of diuresis which varied in the different animals from an output of 6 drops to 17 drops of urine per minute. The systolic blood pressure varied from 100 mm. of mercury to 132 mm. of mercury.

At this period of the experiments the animals were given intravenously 25 c. c. per kilogram of a solution of sodium carbonate equimolecular with 1.5 per cent. solution of sodium chloride. The immediate effect of such injections was to increase the alkali reserve of the blood and to induce a very free flow of urine. The alkali reserve determinations made half an hour after such injections varied from 8.15 to 8.2. The maximum increase in the alkali reserve of the blood which occurred in the animals of Experiments 5 and 6 was from the normal readings of 7.95 and 8.0 to 8.15 and 8.2.

The urine flow in the different animals varied from 24 to 34 drops per minute. The most marked increase occurred in the animal of Experiment 6, in which the normal urine flow was increased from 10 to 31 drops per minute. The systolic blood pressure was raised in all of the animals by the injection of the alkaline solution. The blood pressure in the different animals varied from 100 to 142 mm. of mercury.

By the end of the second half hour of the experiments the reserve alkali of the blood had undergone a marked depletion in all of the animals, and in the animal of Experiment 6 the normal acid-base equilibrium of the blood had been reestablished. The animals continued to remain freely diuretic during this early period of the experiments. There occurred but slight reductions in the systolic blood pressure.

At this stage of the experiments, the end of the second half-hour period, the injection of the alkaline solution was repeated. As was the case from the initial injection of such a solution, the acid-base equilibrium of the blood was disturbed by the increase in its hydroxyl ion content. The maximum increase in the reserve alkali occurred in the animals of Experiments 4 and 6, in which determinations of 8.25 were obtained. The systolic blood pressure in all of the animals was increased.

The results so far obtained from the second injection of a solution of sodium carbonate are similar in character to the effect from the first carbonate injection. The influence, however, which the second injection has on urine formation differs from the previously recorded results. The flow of urine was increased in only one animal of the series. In this animal, Experiment 5, there existed before the beginning of the experiment a reduction in the reserve alkali of the blood. The second carbonate injection in this animal raised the alkali reserve of the blood only to 8.15, and the urine formation increased from 10 to 21 drops per minute. In the remaining animals in which the reserve alkali was increased to, or beyond 8.2, urine formation was either not increased or a reduction in urine formation occurred.

Commencing with the fourth half hour period of the experiments, the results obtained for the remaining two hours have been of the same character for all of the animals.

There is a gradual attempt at a restoration of the normal acid-base equilibrium of the blood. This has not been accomplished by any of these naturally nephropathic animals. The alkali reserve readings at the termination of the experiments varied from 8.0 to 8.2. The reading of 8.0 was obtained in the animal of Experiment 5, in which before the beginning of the experiment the alkali reserve was depleted to 7.95.

Associated with this persisting disturbance in the physico-chemical state of the blood, even though the systemic blood pressure of the animals was well maintained until the end of the experiments, there was a gradual reduction in urine formation. None of the animals became anuric.

Urine formation at the termination of the experiments varied from 1 to 5 drops per minute. Urine collected during the experiments showed a



trace of albumin but no casts. The amount of albumin was apparently not increased during the period of experimentation. In the animal of Experiment 5, in which the reserve alkali was 7.95 prior to the experiment and 8.1 at its termination, the amount of albumin in the urine was reduced. The urine from two of the animals showed a trace of diacetic acid.

The elimination of phenolsulphonephthalein was slightly reduced in all of the animals. This reduction, with one exception, was not in excess of that obtained from a period of anesthesia by Gréhan's anesthetic in naturally nephropathic animals that had not received an alkaline solution. (Table 1. Study II.)

The elimination of the dye varied from a minimum output of 20 per cent. to a maximum output of 40 per cent. The most marked reduction occurred in the animal of Experiment 4, in which the acid-base equilibrium of the blood at the termination of the experiment showed the most marked degree of disturbance. The reserve alkali of the blood at this time was 8.2, whereas the normal reading for this animal was 8.05. The normal elimination of phenolsulphonephthalein by this animal was 50 per cent. At the end of the experiment the output was 20 per cent.

The histological study of the kidneys from these naturally nephropathic animals shows the same type of chronic glomerular pathology that has been previously described. As has been noted in a previous publication,<sup>20</sup> when such animals are given an alkaline solution, the amount of stainable lipid material which can be demonstrated in the renal epithelium is very greatly reduced. The principle changes developing in the kidney take place in the tubular epithelium and mainly in the convoluted tubule cells. In such cells there has developed a moderate grade of cloudy swelling with the nuclei of the cells staining imperfectly. Vacuolation has been occasionally noted. The cells have not shown evidence of necrosis. Fig. 3. Study II.

The most marked changes of this character developed in the kidneys of the animal of Experiment 4, in which there persisted the most marked disturbance in the acid-base equilibrium of the blood and in which the elimination of phenolsulphonephthalein was reduced to 20 per cent. in a two hour period.

*Conclusions Concerning the Effect of a Solution of Sodium Carbonate Equimolecular With a 1.5 Per Cent Solution of Sodium Chloride on Renal Function and Pathology in Naturally Nephropathic Dogs.*

1. When naturally nephropathic animals are given such a solution of sodium carbonate there occurs a disturbance in the acid-base equilibrium of the blood from the introduction of an excess of hydroxyl ions. Following the first injection of such a solution in these animals, in which the chronic kidney injury is largely



confined to the glomeruli, the kidney responds to this degree of departure from the normal in the physico-chemical state of its blood supply by a profuse diuresis in an attempt to restore a normal acid-base equilibrium. This restoration has been accomplished in two of the eight animals employed in the experiments, and in all of the animals the normal alkali reserve of the blood was reduced to very near the normal reading for the respective animal.

2. When the naturally nephropathic animal is subjected to a second injection of such a solution there occurs a second disturbance in the acid-base equilibrium of the blood more marked than that induced by the first injection. Even though the same amount of solution per kilogram of body weight was injected, there developed in only one animal an increase in urine formation. In the remaining animals the flow of urine either remained unchanged or was reduced.

There is, following this second injection of a solution of sodium carbonate, an attempt on the part of the animals to re-establish a normal acid-base equilibrium of the blood, and as a result the reserve alkali is reduced but not to the readings normal for the respective animals.

The normal physico-chemical state of the blood is not re-established and the kidneys reflect this change in their environment by a decrease in their functional response. Urine formation is progressively lessened but not arrested. This decrease in functional response is furthermore shown by a reduction in the elimination of phenolsulphonephthalein.

3. One of the animals in this group had before the experiment a reserve alkali of the blood of 7.95. In this animal the two injections of the alkaline solution induced less disturbance in the acid-base equilibrium of the blood than was obtained in animals with a normal reserve alkali. At the termination of the experiment the reserve alkali of this animal was normal. During the course of the experiment the animal remained freely diuretic and the elimination of phenolsulphonephthalein was reduced only 8 per cent. from the normal.

4. From these observations it would appear that naturally nephropathic animals normally under the strain as a result of

their kidney injury to maintain a normal acid-base equilibrium of the blood, can following one injection of a carbonate solution equimolecular with 1.5 per cent. sodium chloride solution, readjust this equilibrium, in part by a profuse secretion of urine. When, however, such kidneys are subjected to a second strain by such an injection the mechanism through which the readjustment takes place becomes impaired, urine formation is not increased but is reduced, and the acid-base equilibrium of the blood remains disturbed. With such a physico-chemical disturbance persisting, not only is urine formation progressively reduced, but the elimination of phenolsulphonephthalein shows a reduction which is correlated with the degree of disturbance in the acid-base equilibrium of the blood.

5. The histological changes developing in the kidney consist of cloudy swelling, edema, and vacuolation of the tubular epithelium. Such changes have constantly developed in the kidney when its blood chemical environment was sufficiently disturbed by the use of either an acid or an alkaline solution.

*The Effect of a Sodium Carbonate Solution Equimolecular With a 3 Per Cent. Solution of Sodium Chloride on Renal Function and Pathology in Naturally Nephropathic Dogs.*

Ten naturally nephropathic animals were used in this series of experiments. The urine from all of the animals contained both albumin and casts. The elimination of phenolsulphonephthalein by the different animals varied from a minimum output of 30 per cent. to a maximum output of 56 per cent. The reserve alkali of the blood varied from 7.9 to 8.1.

Following the development of a state of anesthesia from Gréhant's anesthetic the animals were given the usual preliminary injection of an isotonic solution of sodium chloride. The degree of diuresis obtained varied from a flow of urine of 3 drops per minute to a maximum flow of 18 drops of urine. The systolic blood pressure in the different animals varied from 98 to 120 mm. of mercury.

After an interval of half an hour the animals were given the first intravenous injection of sodium carbonate solution equimolecular with a 3 per cent. solution of sodium chloride. This resulted in an increase in the alkali reserve of the blood in all of the animals, that varied from 8.2 in the animal of Experiment 8 that had a normal alkali reserve of 7.9, to a reading of 8.35 in the animals of Experiments 11 and 14.

Following this degree of disturbance in the acid-base equilibrium of the blood, there occurred only a transitory increase in urine formation, which was not so marked as was the case in the previously described

group of naturally nephropathic animals that received the weaker carbonate solution. The maximum increase in urine of 12 drops per minute developed in the animals of Experiments 11 and 14.

The introduction of this volume of fluid caused a rise in the systolic blood pressure of all of the animals. This varied from the minimum rise of 10 mm. of mercury in the animal of Experiment 7 to a maximum rise of 24 mm. of mercury in the animal of Experiment 9.

At the end of the second half-hour period of the experiments all of the animals, with the exception of the animal of Experiment 8, had made some attempt to re-establish a normal acid-base equilibrium of the blood. In no instance was this equilibrium restored.

Urine formation at this early stage of the experiments was reduced in all of the animals save two, even though the systolic blood pressure was well maintained and the blood hydremic from the use of the isotonic salt solution and the solution of sodium carbonate.

In two of the animals urine formation was increased. In these animals there had occurred a more marked reduction in the alkali reserve of the blood than in the other animals of the group. In the animal of Experiment 9, the reserve alkali was reduced from 8.3 to 8.1. The normal reserve alkali for this animal was 8.05. In the animal of Experiment 10, the reserve alkali was reduced from 8.3 to 8.15. The normal alkali reserve for this animal was 8.1.

From these observations it would appear that, if the animal is able to readjust the normal acid-base equilibrium of the blood to within the range of the normal, urine formation is increased. If, however, such a readjustment cannot be made, even though the blood of the animal be hydremic and the systolic blood pressure well maintained, urine formation is reduced.

At the end of the second half-hour period of the experiments the animals were given the second injection of the stronger carbonate solution. This injection induced a secondary increase in the reserve alkali of the blood which varied from 8.3 to 8.5.

There occurred a rise in the systolic blood pressure of all of the animals. The blood pressure of the different animals varied from 118 to 136 mm. of mercury.

Following these changes there was a transitory and slight increase in urine formation in two of the animals; no increase occurred in one animal, and in the remaining animals urine formation was reduced.

By the end of the fourth half-hour period of the experiments, one hour after the second injection of the carbonate solution, there was but slight change in the acid-base equilibrium of the blood. The reserve alkali readings varied from 8.2 to 8.4. Those animals in which the reserve alkali of the blood showed the least depletion toward the normal showed the greatest reduction in urine formation. The animal of Experiment 12 was anuric

at this period and two of the animals, Experiments 8 and 11, were forming only 1 drop of urine per minute.

From this stage of the experiments until their termination two hours later, the changes were in general constant for all of the animals. The disturbance in the acid-base equilibrium of the blood was not adjusted. At the conclusion of the experiments the reserve alkali readings varied from 8.1 to 8.35.

Urine formation underwent a progressive reduction, so that at the end of the experiments six of the ten animals were anuric and one of the remaining animals was forming only 1 drop of urine every two minutes. The maximum urine flow was 5 drops per minute.

Those animals in which there persisted the more marked disturbance in the acid-base equilibrium of the blood were the first animals of the group to show a marked reduction in urine formation or to become anuric; while the animals in which the reserve alkali of the blood had been reduced toward the normal were the animals that formed urine to a later period in the experiments or remained diuretic until their termination.

The urine collected during the course of the experiments contained only a trace of albumin and no casts. In five of the animals the amount of albumin was distinctly less than was normal for the nephropathic animal. Diacetic acid was not present in the urine.

In all of the animals the elimination of phenolsulphonephthalein underwent a marked reduction. In three of the animals only a trace of the dye could be detected in the urine. In the remaining animals in which urine formation was sufficient to permit a determination, the elimination varied from 10 to 25 per cent. This observation is of interest when contrasted with other changes in the urine. The amount of albumin in the urine may be reduced to a mere trace and yet the elimination of phenolsulphonephthalein may be so low as not to permit a determination.

The histological changes which developed in these naturally nephropathic animals show an exaggeration of the type of change that has been described as occurring in the animals that received a weaker solution of sodium carbonate.

The same type of chronic glomerular pathology has been observed. No acute exudative or degenerative changes have developed in the glomeruli. The tubular epithelium either shows no stainable lipoid material, or it is present as minute dust-like particles.

The characteristic damage to the kidney occurs in the cells of the convoluted tubules. These cells are severely swollen, vacuolated and undergoing necrosis. Such changes are less marked in those animals that were able to effect some restoration toward the normal in the acid-base equilibrium of the blood. Fig. 4. Study II.

*Conclusions Concerning the Effect of a Solution of Sodium Carbonate Equimolecular With a 3 Per Cent. Solution of Sodium Chloride on Renal Function and Pathology in Naturally Nephropathic Dogs.*

1. A solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium chloride is more toxic for naturally nephropathic animals than is a solution equimolecular with a 1.5 per cent. solution of sodium chloride.

When the stronger solution is given to such animals there occurs a more marked disturbance in the acid-base equilibrium of the blood and with this greater degree of disturbance there is shown less ability on the part of the animals to deplete the reserve alkali and re-establish a normal physico-chemical state of the blood.

2. After a transitory increase in urine formation following such injections, there develops a rapid reduction in urine formation. The persisting and marked change in the character of the blood furnished the kidney so alters its environment that its functional response is reduced very early in the experiments.

3. A second injection of such a solution intrinsifies this disturbance, and following it the organism shows a lessened ability to readjust its acid-base equilibrium toward the normal. Urine formation is further reduced or the animals become anuric, even though the systolic blood pressure of the animals be well maintained and the blood going to the kidney be kept in a hydremic state.

4. This inability of the kidney to functionate with such a disturbance in the acid-base equilibrium of the blood supplied to, is furthermore shown by its inability to eliminate phenolsulphonephthalein.

5. The anatomical changes developing in the kidney under such a changed environment are similar in character, though more extensive in degree, to those changes previously described for the kidneys of naturally nephropathic animals that received a weaker solution of the alkali and which were able more nearly to restore the acid-base equilibrium of the blood to the normal.



*General Discussion of the Effect on Renal Function and Pathology of Inducing a Disturbance in the Acid-Base Equilibrium of the Blood of Both Normal and Naturally Nephropathic Animals by the Introduction of Acid and Alkaline Solutions.*

A review of the results obtained in Studies I and II in which acid and alkaline solutions were administered intravenously to both normal and naturally nephropathic animals not only shows certain variations in the quantitative response of these two types of animals to such solutions, but the observations permit certain conclusions concerning the influence of changes in the acid-base equilibrium of the blood on renal function and pathology. Probably similar changes develop in the functional units of the organism other than the kidneys. The kidney, as was pointed out earlier in these studies, was selected for these observations on account of the ease with which its functional response can be ascertained and for the reason that it is the functional unit of the animal that has most to do with maintaining a normal physico-chemical state of the blood, to which not only the kidney but all other functional units must properly adjust themselves in order to functionate in a normal manner.

When a normal solution, such as isotonic sodium chloride, is given intravenously either to normal or to naturally nephropathic animals there occurs no pronounced diuretic effect even though certain conditions are made more favorable for urine formation. Such a solution produces a more hydremic blood, the viscosity of the blood is decreased and there is a rise in systolic blood pressure.

When abnormal solutions, such as solutions of hydrochloric acid or sodium carbonate, are given to normal or to naturally nephropathic animals a change in the blood chemical environment of the animal is induced, and there is at once thrown into operation a mechanism, the kidney, which attempts by a great increase in its functional response to restore the normal physico-chemical state of the blood in so far as the normal acid-base equilibrium of the blood is concerned.

In normal animals, as such a restoration is effected, there occurs a decrease in urine formation which is not due to a renal injury induced by the solutions. At such a time, when urine formation

is decreased, the elimination of phenolsulphonephthalein by the kidney is but slightly interfered with. If the use of such solutions be repeated and the acid-base equilibrium of the blood again disturbed, the kidney responds to this further change in its environment by another attempt at readjustment. In this second response even the normal kidney has been found to be inadequate. The disturbed physico-chemical state of the blood is not readjusted with sufficient rapidity or to a proper degree to enable the kidney to cope with its environment, and this lack of adjustment is expressed functionally by a decrease in urine formation or the development of an anuria, by the appearance of albumin in the urine, and by a greatly reduced elimination of phenolsulphonephthalein.

The pathological response on the part of the kidneys of both normal and naturally nephropathic dogs to such an alteration in their blood chemical environment has with one difference been the same, irrespective whether the changed environment was due to an excess of hydorgen or hydroxyl ions. The changes in the kidney can not therefore be ascribed to any specific influence of acid or alkaline solutions.

These changes consisted in edema and vacuolation of the renal epithelium and particularly of the specialized cells which line the convoluted tubules.

The necrotic changes in the epithelium varied in the different animals. Those animals that effected the least restoration toward the normal in the acid-base equilibrium of the blood were the animals in which the most marked epithelial degeneration occurred, regardless of whether an acid or an alkaline solution was employed.

The difference in the pathological response of the kidneys of these animals to acid or alkaline solutions was associated with the amount of stainable lipid material which can be demonstrated in the renal epithelium. This difference does not depend upon the degree of the disturbance in the acid-base equilibrium of the blood, but is associated with the character of the disturbance, whether it be due to an excess of hydrogen or hydroxyl ions. If the disturbance was induced by the introduction of an acid solution, the amount of stainable lipid material is very

greatly increased in the cells of the loops of Henle and furthermore appears in the convoluted tubule epithelium. If, on the other hand, the change in the acid-base equilibrium of the blood was induced by the use of an alkaline solution, there is a decrease or absence of such stainable lipoid in the cells of the loops of Henle and in the cells of the convoluted tubules.

In the studies that have been made in naturally nephropathic animals of the disturbance in the acid-base equilibrium of the blood from the use of acid or alkaline solutions a similar type or quality of response was obtained, though the degree of disturbance was in excess of that observed in normal animals.

In six of the naturally nephropathic animals, the kidneys were unable prior to any experimental interference to maintain a normal acid-base balance of the blood. When a solution of hydrochloric acid is administered to such an animal normally under the strain as a result of the chronic nephropathy of maintaining a normal acid-base equilibrium of the blood, the solution induces a more marked disturbance in this physico-chemical state of the blood than is induced in a normal animal.

The kidney responds to this changed environment by an increase in urine formation, which in turn is not so marked as that which occurs in a normal animal. The restoration of the acid-base equilibrium of the blood is not accomplished to the same degree as occurs in normal animals. The changed environment persists, an adaptation of the kidney to this environment in terms of its functional response is not made, and urine formation is reduced. When such a solution is again introduced into a naturally nephropathic animal, there occurs a further departure from the normal in the blood chemical environment of the kidney, and urine formation is usually rapidly reduced and a state of anuria established.

When alkaline solutions are given intravenously to naturally nephropathic animals, the results obtained depend upon two factors: first, the molecular concentration of the solution; and second, whether or not at the time of the use of the solution the animal was able to maintain a normal alkali reserve of the blood.

When solutions of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride are administered to naturally

nephropathic animals with a normal acid-base equilibrium of the blood, there occurs following the disturbance induced in this equilibrium a free diuresis. The kidney either effects a restoration of its normal blood chemical environment or a restoration is established to such an extent that the kidney remains uninjured.

When a second injection of such a solution is employed, the disturbance in the acid-base equilibrium of the blood is more marked than occurred from the first injection. The change in environment is so great that the kidney can not effect a rapid readjustment, and the inability to make this adjustment is shown by a decrease in urine formation, which may go to the extent of the establishment of a state of anuria. When, however, such a solution of sodium carbonate is given to naturally nephropathic animals with a decrease in the reserve alkali of the blood below the normal, the result is either to re-establish a normal blood chemical environment for the kidney to functionate in, or to disturb this environment to a less degree than is induced when such a solution is given to an animal with a normal acid-base equilibrium of the blood. In such animals the functional response of the kidney is improved, urine formation is more marked, and the elimination of phenolsulphonephthalein is reduced to a less extent.

The response of naturally nephropathic animals to intravenous injections of a solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium chloride is of the same general character as that outlined when the weaker solution of the alkali is given to such animals. The disturbance in the acid-base equilibrium of the blood is more marked from such a solution, so that even from the first injection only a slight diuretic effect may be obtained. The blood chemical environment of the kidney remains severely altered.

When the second injection of the stronger carbonate solution is employed, with the kidney even at this early stage unable to readjust its environment, there occurs in most of the animals a further reduction in urine formation, a more marked disturbance in the physico-chemical state of the blood, a very great reduction or a practical absence of the elimination of phenolsulphonephthalein, and in the majority of the animals an anuria is established.

The pathological response of the naturally nephropathic kidney to the alkaline solutions of different molecular concentration has been of the same type as has occurred from the use of acid solutions, with the exception noted in connection with the amount of stainable lipoid material that can be demonstrated in the renal epithelium. These changes have been very largely localized in the convoluted tubule epithelium, and consisted of an edema with vacuolation and necrosis of these cells. The degree to which these changes develop depends upon the duration and severity of the disturbance in the blood chemical environment of the kidney.

#### REFERENCES.

##### STUDY II.

1. Ewing, James. *Arch. Int. Med.*, 2, 1908, 330.
2. Sellards, A. W. *Harvard University Press, Cambridge*, 1917.
3. Von Hösslin, R. *Munch. med. Woch.*, 56, 1673, 1909, 1673.
4. Fischer, Martin H. *Trans. Assn. Amer. Phys.*, 27, 1912, 595.
5. Palmer, W. W., and Henderson, L. J. *Arch. Int. Med.*, 12, 1913, 153.
6. Palmer, W. W., and Henderson, L. J. *J. Biol. Chem.*, 21, 1915, 57.
7. Peabody, F. W. *Arch. Int. Med.*, 16, 1915, 955.
8. Chase, Arthur F., and Myers, Victor C. *J. Amer. Med. Assn.*, 74, 1920, 641.
9. MacNider, Wm. deB. *J. Exp. Med.*, 26, 1917, 1.
10. MacNider, Wm. deB. *Science, N. S.*, 53, 1921, 141.
11. MacNider, Wm. deB. *J. Exp. Med.*, 28, 1918, 501.
12. MacNider, Wm. deB. *J. Exp. Med.*, 28, 1918, 517.
13. MacNider, Wm. deB. *J. Exp. Med.*, 29, 1919, 513.
14. MacNider, Wm. deB. *Arch. Int. Med.*, 26, 1920, 1.
15. Pearce, R. M. *Med. Bull. Univ. Penn.*, 16, 1903-4, 217.
16. Ophüls, W. *J. Med. Research*, 18, 1908, 497.
17. Dayton, H. *J. Med. Research*, 31, 1914, 177.
18. MacNider, Wm. deB. *J. Med. Research*, 34, 1916, 177.
19. MacNider, Wm. deB. *J. Med. Research*, 34, 1916, 199.
20. MacNider, Wm. deB. *J. Pharm. and Exp. Therap.*, 20, 1922, 365.



## DESCRIPTION OF FIGURES.

## STUDY II.

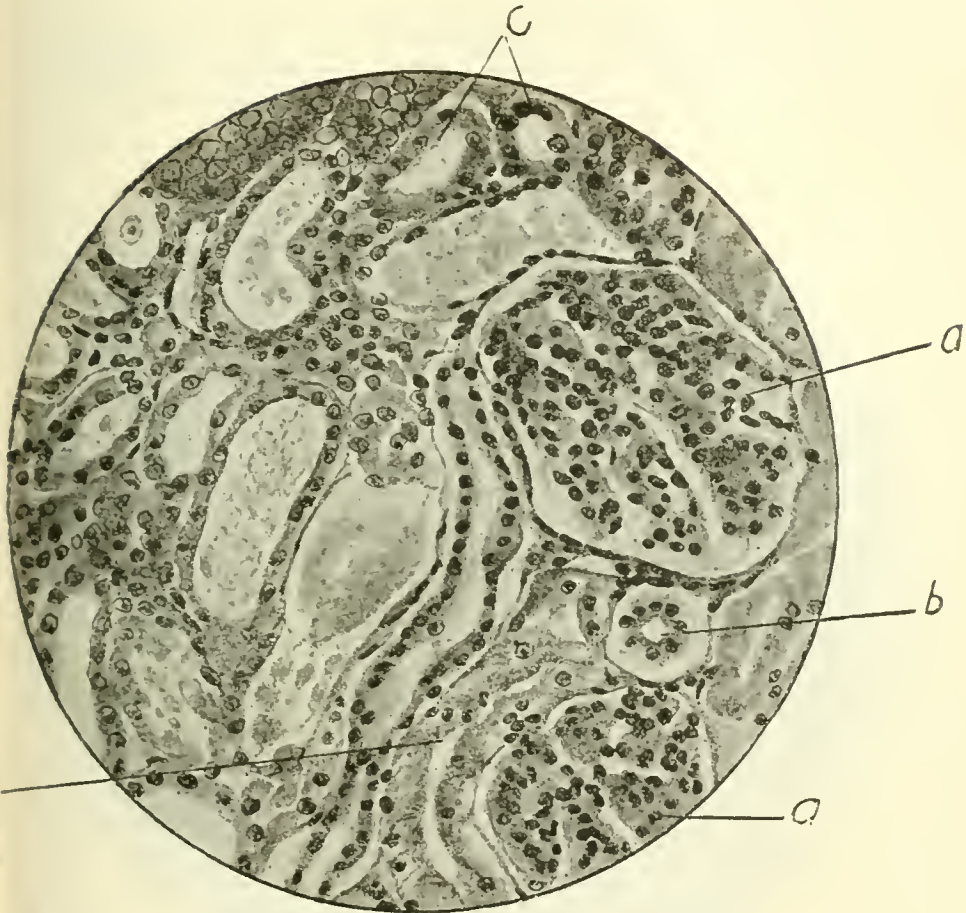


Fig. 1. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the kidney of the naturally nephropathic control animal of Experiment 1, Study II, Table 1. The animal was anesthetized by Gréhant's anesthetic for a period of four hours. With the completion of a satisfactory state of anesthesia, the animal was given intravenously 25 c. c. per kilogram of a 0.9 per cent. solution of sodium chloride. The animal did not receive either an acid or an alkaline solution during the experiment. An anesthesia of this duration with Gréhant's anesthetic in a naturally nephropathic animal has resulted in a decrease in urine formation so that at the end of the experiment only two drops of urine per minute was formed. The reserve alkali of the blood was reduced from

the normal of 8.0 to 7.9. The amount of albumin in the urine was increased. The elimination of phenolsulphonephthalein was reduced from the normal of 56 per cent. to 35 per cent.

At A, are shown glomeruli with an increase in nuclei and an obliteration of the capillary loops. The capillary mass is lobulated and adherent in places to the capsule. At B, are shown convoluted tubules with edematous epithelium. At C, are other tubules in which the swelling is less marked.

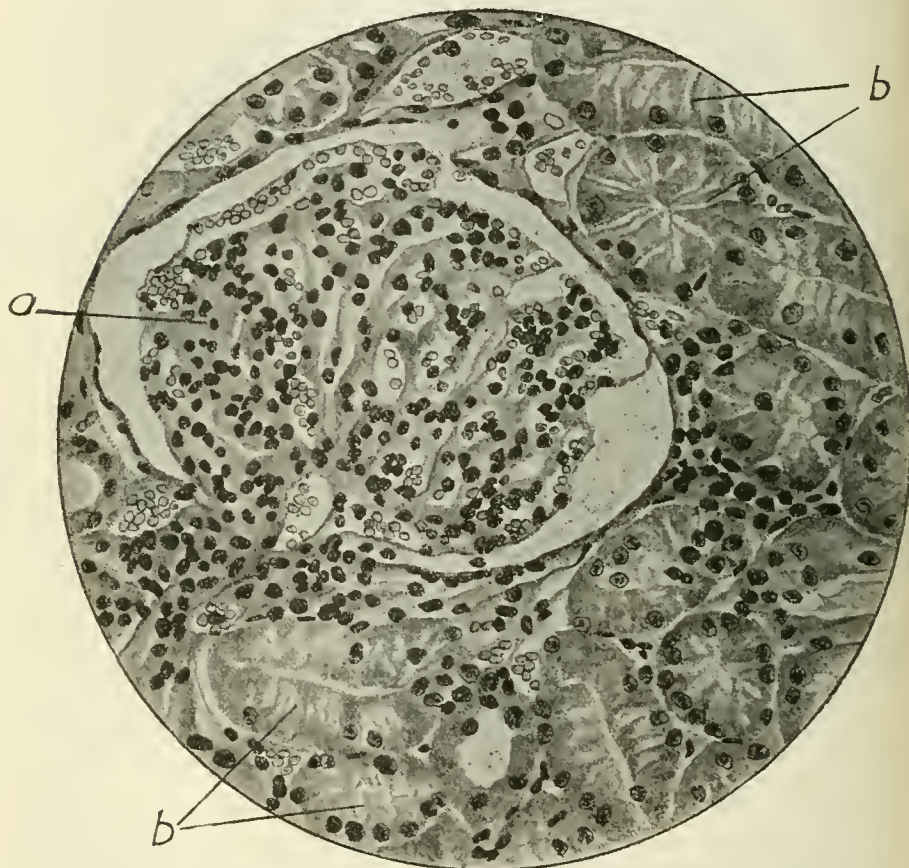


Fig. 2. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the kidney of the naturally nephropathic animal of Experiment 15, Study II, Table 1.

The animal received two injections of N/2 hydrochloric acid. The animal was unable to re-establish a normal acid-base equilibrium of the blood. The reserve alkali of the blood was reduced from the normal reading of 8.0 to 7.6. Early in the experiment a heavy precipitate of

albumin appeared in the urine. Numerous casts were present. The elimination of phenolsulphonephthalein was reduced from 46 per cent. to a mere trace. Early in the experiment the animal became anuric.

At A, is shown a large glomerulus. The walls of the capillaries are greatly thickened. At one place the loops are adherent to the thickened capsule. At B, are shown convoluted tubules severely swollen, vacuolated and in an early stage of necrosis.

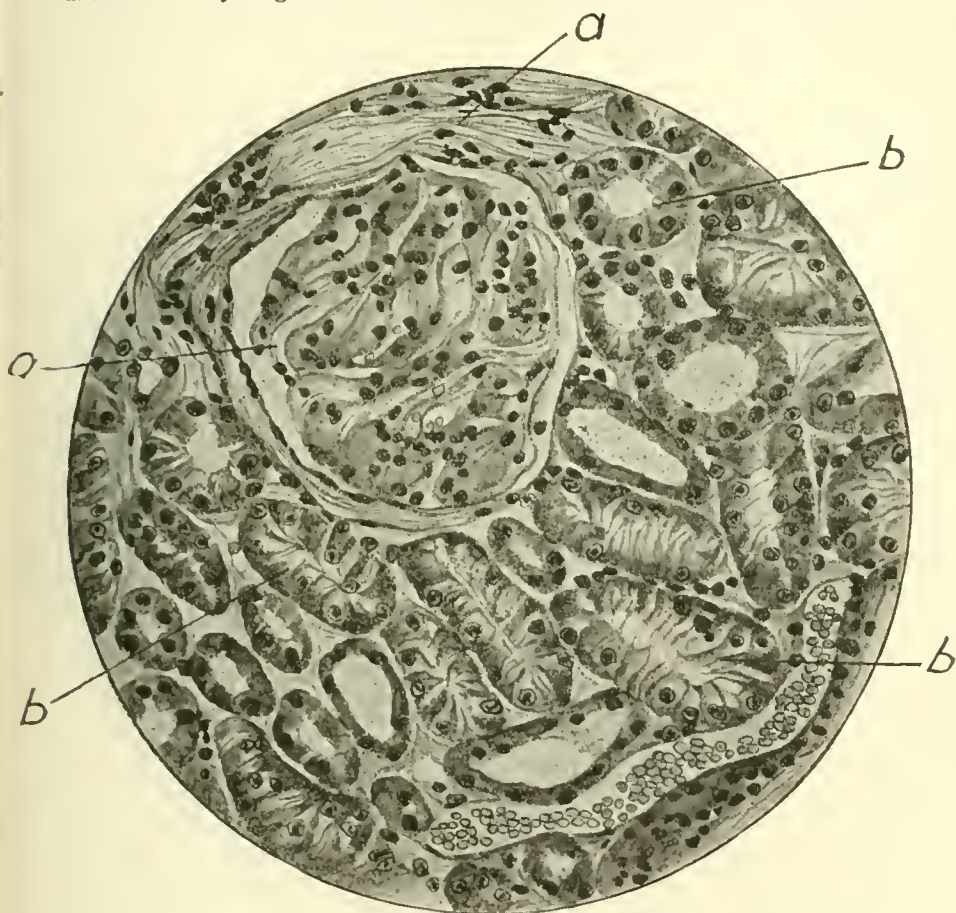


Fig. 3. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the kidney of the naturally nephropathic animal of Experiment 2, Study II, Table 2.

The animal received two intravenous injections of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride. The animal succeeded in a partial restoration of the acid-base equilibrium of the blood. At the termination of the experiment the reserve alkali was



8.1 as opposed to the normal reserve alkali of 8.0. The flow of urine was 5 drops per minute. The urine was free from both albumin and casts. The elimination of phenolsulphonephthalein was only reduced from the normal of 42 per cent. to 38 per cent.

At A, is shown a severely fibrosed glomerulus with a thickened capsule and a periglomerular fibrosis. At B, are shown convoluted tubules with a variable amount of swelling of the cells. The nuclei in general stain well. The cells are not necrotic.

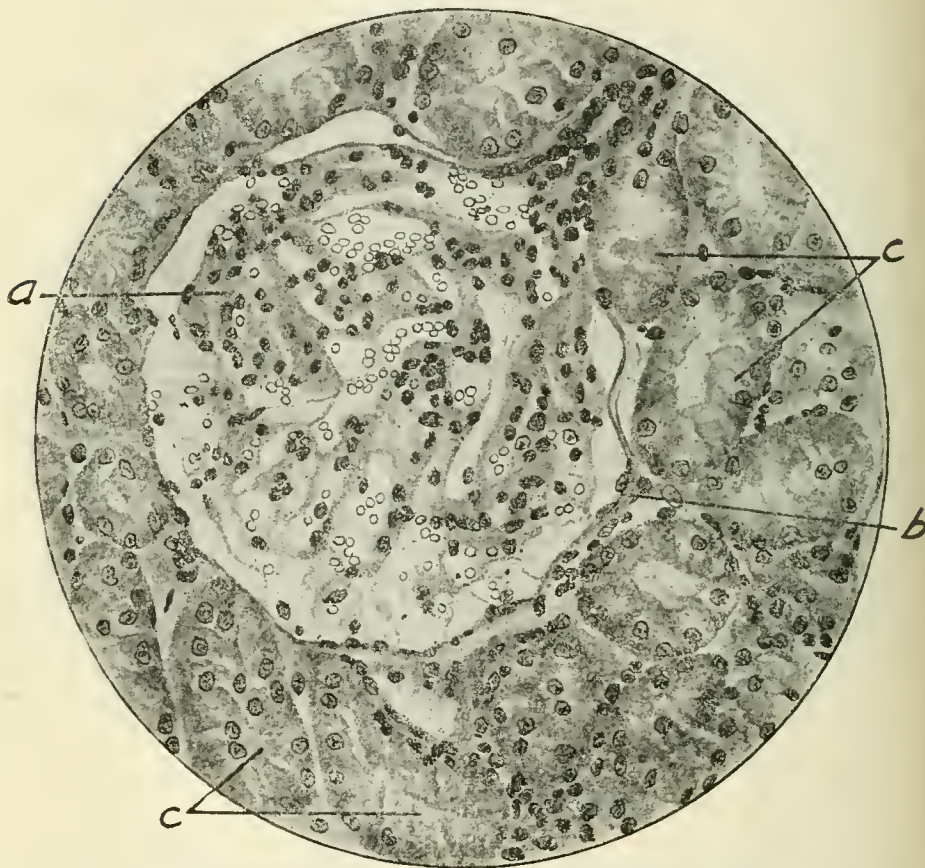


Fig. 4. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the kidney of the naturally nephropathic animal of Experiment 14, Study II, Table 2.

The animal received two intravenous injections of a solution of sodium carbonate equimolecular with a 3 per cent. solution of sodium chloride. The animal was unable to re-establish a normal acid-base equilibrium of the blood. At the termination of the experiment the reserve alkali was

8.3, as opposed to the normal reserve alkali of 8.05. The animal during the course of the experiment became anuric. Before the establishment of the anuria the urine contained albumin but no casts. Phenolsulphonephthalein appeared as a trace.

At A, is shown a large glomerulus. The capillary walls are greatly thickened. The capillary loops are adherent to the capsule which is only slightly thickened. At B, are shown convoluted tubules. The epithelium is edematous and vacuolated and shows in some tubules a well advanced necrosis.





STUDIES CONCERNING THE INFLUENCE OF A  
DISTURBANCE IN THE ACID-BASE EQUILI-  
BRIUM OF THE BLOOD ON RENAL  
FUNCTION AND PATHOLOGY.

STUDY III. THE ABILITY OF AN ALKALINE SOLUTION TO  
PROTECT THE KIDNEY OF NORMAL AND NATURALLY  
NEPHROPATHIC DOGS AGAINST AN ACID SOLUTION.

Wm. deB. MacNider.

*The Laboratory of Pharmacology, University of North Carolina.*

Sellards<sup>1</sup> in one of his earlier studies observed the value of sodium bicarbonate in the acidosis associated with the uraemia of cholera and in nephritis. At a date later than this studies<sup>2, 3</sup> conducted in this laboratory demonstrated that the toxicity of uranium nitrate for the kidney was in part dependent upon the degree of disturbance this substance was able to induce in the acid-base equilibrium of the blood. The investigations furthermore demonstrated that if animals were protected against this disturbance in the physico-chemical state of the blood by the use of a solution of sodium carbonate, various diuretic solutions were more effective in such acutely nephropathic animals than they were in control animals that had not received the protection. More recent studies<sup>4, 5</sup> relative to the toxic effect of the general anesthetics for the normal and naturally nephropathic kidney have shown a relationship to exist between the degree of disturbance induced by the anesthetic in the acid-base equilibrium of the blood with the toxicity of such substances for the kidney. These studies furthermore demonstrated the ability of a solution of sodium carbonate to protect the normal kidney and, to a less extent, the naturally nephropathic kidney against such an injury.

In an investigation<sup>6</sup> of a similar nature, the observation was made that if a solution of sodium carbonate be given acutely nephropathic animals before the commencement of an anesthetic the kidneys are protected against the toxic effect of the anesthetic substance. This observation was at a later date confirmed by Goto.<sup>7</sup> Very recently Hara<sup>8</sup> has shown the value of a diet which induces an alkaline urine in protecting the kidneys of rabbits against injury.

\* Aided by a grant from the Rockefeller Institute for Medical Research.

In the preceding investigations, Studies I and II, the observation has been made that when the blood chemical environment of the kidney of normal and naturally nephropathic animals was changed to a sufficient degree by a disturbance in the acid-base equilibrium of the blood, the kidney was unable to readjust its environment, a definite type of renal injury was induced, and the functional response of the kidney was decreased or suspended.

These studies have shown that the intravenous injection of 5 cc. per kilogram of a N/2 solution of hydrochloric acid in normal dogs induces such a change in the physico-chemical state of the blood that renal function is very greatly reduced. When such solutions are administered to naturally nephropathic animals, either a more marked interference with renal function occurs or an anuria develops. A further observation has been made in these experiments: that one intravenous administration of 25 cc. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride does not alter the environment of the kidney to the extent that it can not effect a readjustment of the acid-base equilibrium of the blood to within normal bounds. The use of such a solution does not decrease the functional response of the kidney.

With these observations in mind, the following investigation was undertaken to ascertain the ability of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride to protect both the normal and naturally nephropathic kidney against the toxic effect of a N/2 solution of hydrochloric acid.

Ten normal and eight naturally nephropathic dogs were used in these experiments. Five of the normal animals served for control experiments. Such animals were given the solution of hydrochloric acid but did not receive prior to such an injection a solution of sodium carbonate. The remaining animals were first given the alkaline solution and at a later period the acid solution.

Of the eight naturally nephropathic animals four were used for control experiments. They were given the acid solution but not the alkaline solution. The remaining animals were first given the solution of sodium carbonate and half an hour later a N/2 solution of hydrochloric acid. The results obtained in six of the normal animals and in six of the naturally nephropathic animals were included in Table I, Study I. The experimental technique employed in these experiments was identical with that used in the previous studies.

*The Ability of an Alkaline Solution to Protect the Kidneys of Normal Dogs Against an Acid Solution.*

The preliminary study of these animals showed them to have a normal urine. The elimination of phenolsulphonephthalein varied from 60 to 81 per cent. The reserve alkali of the blood varied from 8.0 to 8.1.

The control experiments 1, 4 and 6, Table I, show the effect of a N/2 solution of hydrochloric acid in normal animals that had not had the protection of an alkaline solution. Experiments 2, 5 and 7 show the results obtained in such animals that have received an alkaline solution prior to the use of the solution of hydrochloric acid.

Following the development of an anesthesia from Gréhan's anesthetic, the animals were as usual given 25 cc. per kilogram of isotonic sodium chloride solution. The control animals of this normal group, Experiments 1, 4 and 6, developed a diuresis which varied from 10 to 21 drops of urine per minute. The systolic blood pressure varied from 100 to 121 mm. of mercury. The reserve alkali of the blood remained unchanged.

At the end of the first half-hour period of the experiments the animals were given 5 cc. per kilogram of a N/2 solution of hydrochloric acid. The use of the solution induced a reduction in the reserve alkali of the blood which varied from a minimum depletion of 7.9 to a maximum depletion of 7.85. Associated with this change in the acid-base equilibrium of the blood, it was observed that the animals became very freely diuretic. The flow of urine varied in the respective animals from 22 to 30 drops per minute. In three of the animals no attempt at a restoration of the reserve alkali of the blood was observed. In the remaining animals the depleted reserve alkali was restored toward the normal. By the end of the fourth half-hour period of the experiments the reserve alkali of the blood had increased in all of the animals, but in none did the readings return to the normal. At this stage of the experiments, when the animals had been unable to readjust their acid-base equilibrium, a marked reduction in urine formation developed which varied from an output of 14 drops of urine per minute by the animal of Experiment 6 to an output of 2 drops per minute by the animal of Experiment 1. The systolic blood pressure in these control animals varied from 100 to 124 mm. of mercury.

A study of the further course of the experiments showed no increase in the reduction of the alkali reserve of the blood. There is a continued inability on the part of the animals to restore the depleted reserve alkali to the normal. During this period urine formation decreased, so that at the termination of the experiments one of the animals was anuric. The maximum flow of urine was 2 drops per minute. The systolic blood pressure for the different animals varied from 102 to 118 mm. of mercury.

A study of the urine formed by the control animals during the experiments shows that the use of the acid solution without protection against it by the use of an alkali leads to the development of an albuminuria with casts. Diacetic acid was present in the urine of two of the animals. The



elimination of phenolsulphonephthalein was reduced during the course of all of the experiments. The output of the dye in a two hour period varied from 33 to 40 per cent.

The histological study of the kidneys of the control animals failed to show any injury to the glomeruli. The epithelium, especially that of the convoluted tubules, showed cloudy swelling and edema. Vacuolation of these cells was not frequent or uniform and only occasionally were necrotic changes observed. Fig. I. Study III.

The results obtained in normal dogs protected against the acid solution by a preliminary administration of one injection of a solution of sodium carbonate are represented in Table I, by Experiments 3, 5 and 7.

Following an interval of half an hour after the development of an anesthesia and the usual intravenous injection of isotonic sodium chloride solution, these animals were given by vein 25 cc. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride.

The reserve alkali of the blood in the different animals increased from the normal readings of 8.0 to 8.1 before the experiments to readings after the use of the alkaline solution which varied from 8.15 to 8.25. The systolic blood pressure in the different animals varied from 126 to 146 mm. of mercury. Immediately following this disturbance in the acid-base equilibrium of the blood a profuse diuresis developed in all of the animals. The flow of urine varied from a minimum output of 31 drops of urine per minute by the animal of Experiment 5 to a maximum flow of urine of 63 drops per minute by the animal of Experiment 7.

At this stage of the experiments the animals were given intravenously 5 cc. per kilogram of a N/2 solution of hydrochloric acid. Such injections resulted in a sudden reduction of the alkali reserve of the blood and in two of the animals were followed by a reestablishment of the normal acid-base equilibrium. Following this change, at the end of the second half-hour of the experiments, the animals continued freely diuretic even though the systolic blood pressure had undergone a reduction in all but two of the animals.

From this stage of the experiments until their termination at the end of the four hour period the results have been very uniform in the different animals. As the experiments progress there occurs a gradual reduction in urine formation. None of the animals became anuric. At the end of the experimental period the flow of urine in the different animals has varied from 11 to 18 drops per minute. The reserve alkali of the blood at the end of the experiments has been within the normal for all of the animals. The reserve alkali has varied from 8.0 to 8.05.

Urine collected during the experiments has been variable as to the presence of albumin and casts. Two of the animals had a urine which showed a trace of albumin but no casts. The urine from the remaining animals was free from both albumin and casts. Diacetic acid was not present.



The elimination of phenolsulphonephthalein was reduced from the normal readings obtained prior to the experiments, but the reduction was much less than occurred in the control animals that did not receive an alkali. The elimination of the dye by the different animals varied from a minimum output of 55 per cent. to a maximum output of 65 per cent.

The histological changes in the kidneys of these animals that have been protected against the toxic effect of a solution of hydrochloric acid by the use of a solution of sodium carbonate are negative in character when compared with the results obtained in the control group of animals. The glomeruli appear normal. The tubular epithelium is shrunken. The cytoplasm stains well and shows a moderate degree of granulation. Vacuolation and necrosis of the cells was not observed. Fig. 2. Study III.

*Conclusions Concerning the Ability of an Alkaline Solution to Protect the Kidney of Normal Dogs Against an Acid Solution.*

1. The intravenous injection of 5 cc. per kilogram of a N/2 solution of hydrochloric acid in normal dogs induces a disturbance in the acid-base equilibrium of the blood which the animal is unable to readjust in a four hour period.

2. Associated with this disturbance in the physico-chemical state of the blood, urine formation is gradually decreased or the animals become anuric. Albumin and casts appear in the urine and the elimination of phenolsulphonephthalein is markedly reduced. The kidneys of such animals show the usual histological changes common to such a disturbance in their environment. The epithelium of the tubules is edematous and vacuolated and more rarely shows an early necrosis.

3. When normal animals are given 25 cc. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride and at a later period of the experiment given a similar amount per kilogram of a solution of hydrochloric acid as was given to the control animals, the animals receiving the alkaline solution are in part protected against the toxic effect of the acid solution.

4. The use of such an alkaline solution decreases the degree of disturbance in the acid-base equilibrium of the blood which is induced by an acid solution. Such animals are able to restore the acid-base equilibrium of the blood to within normal readings. With this lack of departure in the blood chemical environment of the kidney from the normal, urine formation continues

throughout the experiments, either no albumin appears in the urine or it occasionally appears as a trace. The elimination of phenolsulphonephthalein is but slightly reduced.

5. The histological study of the kidneys of such animals shows anatomical evidence of protection. The epithelium of the tubules is not edematous or vacuolated as is the case with such cells that have been subjected to a changed environment from the use of an acid solution without the protection of an alkali.

*The Ability of an Alkaline Solution to Protect the Kidney of Naturally Nephropathic Dogs Against an Acid Solution.*

Eight naturally nephropathic animals were used in these experiments. The urine from all of the animals contained both albumin and casts. The elimination of phenolsulphonephthalein varied in the respective animals from 34 to 52 per cent. The reserve alkali of the blood varied from 7.9 to 8.05.

The results obtained from a study of this group of animals are represented by six experiments included in Table I, Study III. The animals of Experiments 1, 5 and 8 served in the capacity of controls. These dogs received one injection of a N/2 solution of hydrochloric acid without a preliminary injection of an alkaline solution. The animals of Experiments 4, 6 and 9 were first given intravenously a solution of sodium carbonate and at a later period in the experiments an acid solution.

Following the development of an anesthesia from Gréhan's anesthetic the naturally nephropathic animals were given intravenously 25 cc. per kilogram of a 0.9 per cent. solution of sodium chloride. Urine formation by these animals varied from 4 to 11 drops per minute. The naturally nephropathic animals were less responsive to the diuretic effect of such a solution than was the case with the former group of normal animals. The systolic blood pressure in the naturally nephropathic dogs has varied from 105 to 130 mm. of mercury.

The control animals of this group were now given 5 cc. per kilogram of a N/2 solution of hydrochloric acid.

The reserve alkali of the blood was reduced to readings which varied from 7.8 to 7.9. In the animal of Experiment 1, in which the reserve alkali was only reduced from the normal of 8.0 to 7.9, there was an initial increase in urine formation from 4 drops per minute to six drops per minute. In the animal of Experiment 5, in which the reserve alkali was reduced from 7.95 to 7.8, the animal became anuric. In the remaining animals urine formation was reduced. There was no attempt by any of the naturally nephropathic animals to restore the normal acid-base equilibrium of the blood. On the contrary, during the course of the experiments the reserve alkali of the blood underwent a progressive depletion.

At the termination of the experiments the reserve alkali readings for the different animals varied from the low reading of 7.7 to 7.8.

Associated with the continuation of this disturbance in the physico-chemical state of the blood, urine formation rapidly decreased in all of the animals so that by the end of the third hour of the experiments all of the animals were anuric. At this period of the establishment of an anuria the systolic blood pressure for the respective animals varied from 105 to 110 mm. of mercury.

The results obtained in this control group of naturally nephropathic animals differ only in degree from those obtained in the group of control normal animals. The normal animals that received a solution of hydrochloric acid were able either to restore the acid-base equilibrium of the blood toward the normal or to maintain it at a reading not below 7.9. These animals showed a reduction in urine formation. With this degree of disturbance in the environment of the kidney, this unit was still able to functionate to a lessened degree. In the naturally nephropathic animals the use of such an acid solution causes a greater disturbance in the environment of the kidney, and the primarily damaged organ is unable to establish through increased function a restoration of the normal physico-chemical state of the blood. This inability is expressed by a rapid decrease in function and finally by its arrest.

The urine collected during these experiments contained both albumin and casts. The elimination of phenolsulphonephthalein was so greatly reduced that a quantitative determination of the output was impossible. The time of the appearance of the dye was greatly delayed, and its output was only a trace.

The histological study of the control naturally nephropathic animals shows in general the same type of chronic glomerular pathology described in the previous studies. The glomeruli show no evidence of acute degenerative changes. The tubular epithelium of the kidney is the tissue which shows the effect of the physico-chemical change that the acid solution has induced in the blood. These cells, and especially those of the convoluted tubules, show a marked edema and vacuolation and the cells of many of the tubules show an advanced necrosis. Fig. 3. Study III.

The results obtained in naturally nephropathic dogs protected against an acid solution by the preliminary administration of one injection of a solution of sodium carbonate are represented in Table I, by Experiments 4, 6 and 9.

Following the development of a state of anesthesia, the animals were given intravenously the usual solution of isotonic sodium chloride. Urine formation by the different animals varied from 4 to 7 drops per minute. The systolic blood pressure varied from 112 to 130 mm. of mercury. Determinations of the reserve alkali of the blood varied from 7.9 to 8.0.

At the end of the first half-hour period of the experiments the animals were given 25 cc. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride. The reserve

alkali of the blood was increased in all of the animals, but the degree of disturbance was not so marked as was the case in the animals with a normal alkali reserve. These determinations for the group of naturally nephropathic animals following the use of the alkali varied from a reading of 8.1 in the animal of Experiment 5 to a reading of 8.2 in the animal of Experiment 9.

Associated with this change in the acid-base equilibrium of the blood, urine formation increased in all of the animals. The degree of increase was not so great as was the case with the normal animals. The flow of urine varied from a minimum output of 26 drops per minute by the animal of Experiment 4 to a maximum output of 31 drops by the animal of Experiment 9. The systolic blood pressure in the different animals varied from 128 to 138 mm. of mercury.

At this stage of the experiments the animals were given intravenously 5 cc. per kilogram of a N/2 solution of hydrochloric acid. The reserve alkali of the blood was reduced in all of the animals by such an injection, but not to a point below the normal. The alkali reserve readings for the respective animals varied from 8.0 to 8.1. Associated with a failure of the acid solution to reduce the alkali reserve of the blood below the normal, urine formation continued and in only two of the animals was there any reduction in urine formation. At this stage of the experiments with the control animals that had not received an alkaline solution urine formation was reduced, or the animals had become anuric.

During the remainder of the experiments, these naturally nephropathic animals that had received the solution of sodium carbonate continued to form urine. At the conclusion of the experiments urine formation by the different animals varied from 8 to 14 drops per minute.

As the experiments progressed the reserve alkali of the blood underwent a reduction. This change was much less marked than was the case with the control animals of the group that had not received the alkaline solution. The reserve alkali determinations at the end of the experiments varied from 7.9 to 8.0. The systolic blood pressure in the different animals varied from 105 to 120 mm. of mercury. Urine collected during the course of the experiments showed a trace of albumin and no casts. Diacetic acid was present in the urine of three of the animals.

The elimination of phenolsulphonephthalein was reduced to a greater extent than was the case with the normal animals protected by the use of an alkali, but the elimination was greater than the elimination by the naturally nephropathic animals that did not have this protection. The output of the dye in a two hour period varied from 22 to 30 per cent.

A study of the kidneys of this group of naturally nephropathic animals that received an alkaline solution prior to the use of a solution of hydrochloric acid show anatomical evidence of protection in that there is less edema and vacuolation of the epithelium, and necrotic changes in these cells are rarely observed. Fig. 4. Study III.



*Conclusions Concerning the Ability of an Alkaline Solution to  
Protect the Kidneys of Naturally Nephropathic Dogs  
Against an Acid Solution.*

1. The naturally nephropathic kidney is more susceptible to the toxic effect of a N/2 solution of hydrochloric acid than is the normal kidney. Such a solution in naturally nephropathic animals induces a more marked disturbance in the acid-base equilibrium of the blood. The naturally nephropathic animal is unable to readjust this disturbance and establish a normal physico-chemical state of the blood.

2. As a result of the persistence of such a disturbed environment renal function is rapidly reduced, albumin and casts increase in the urine, the elimination of phenolsulphonaphthalein is delayed and decreased in its output to a trace. The animals become anuric.

3. The intravenous injection of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride before the use of such an acid solution confers a partial protection to the kidneys in such animals. The protection is shown by the naturally nephropathic animals being more nearly able to maintain a normal acid-base equilibrium of the blood. With the physico-chemical state of the blood more nearly approaching the normal, the naturally nephropathic animals that have received the alkaline solution continue to form urine until the termination of the experiments. The urine from such protected animals has continued only a trace of albumin and no casts. The elimination of phenolsulphonaphthalein is in excess of that obtained from the animals without the protection. The histological evidence of injury to the kidney is less marked than is the case in naturally nephropathic animals that have not been protected by the use of an alkaline solution.

REFERENCES.

STUDY III.

1. Sellards, A. W. *Bull. Johns Hopkins Hospital*, 23, 1912, 289.
2. MacNider, Wm. deB. *J. Exp. Med.*, 26, 1917, 1.
3. MacNider, Wm. deB. *J. Exp. Med.*, 26, 1917, 19.
4. MacNider, Wm. deB. *J. Exp. Med.*, 28, 1918, 501.
5. MacNider, Wm. deB. *J. Exp. Med.*, 28, 1918, 517.
6. MacNider, Wm. deB. *J. Exp. Med.*, 23, 1916, 171.
7. Goto, R. *J. Exp. Med.*, 27, 1918, 413.
8. Hara, M. *Mitt. a. d. med. Fak. k. Univ. Tokio*, 25, 1920, 1.



## DESCRIPTION OF FIGURES.

## STUDY III.

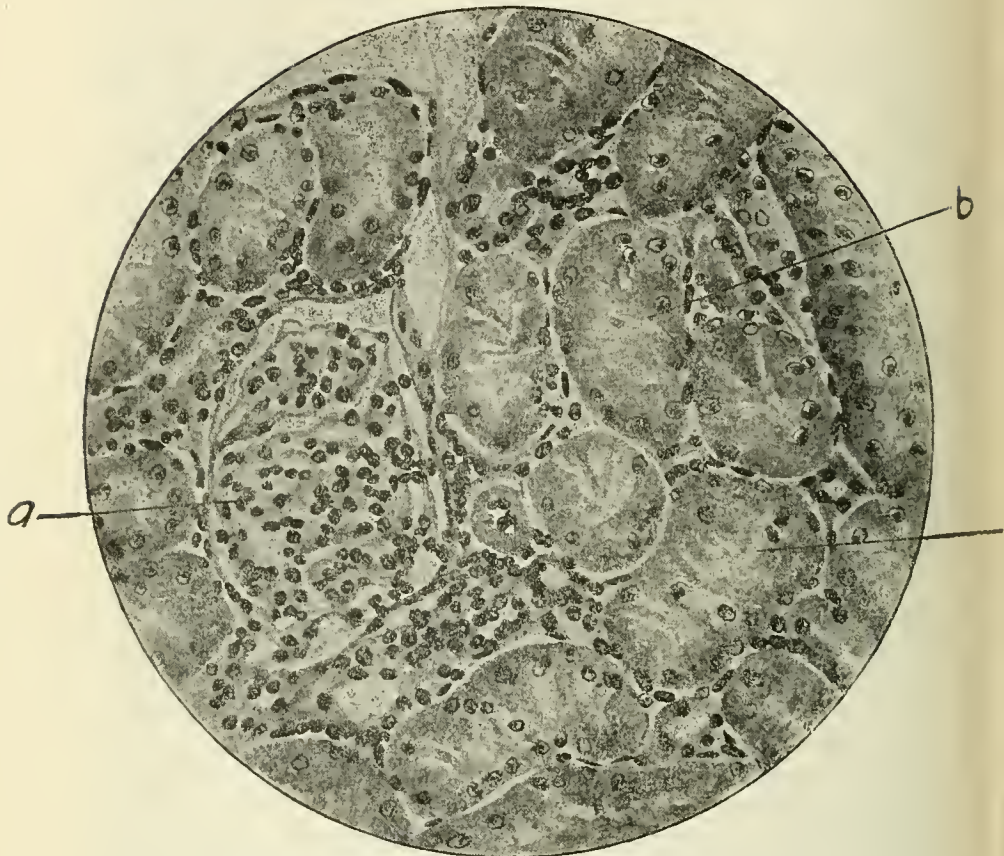


Fig. 1. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the normal control animal of Experiment 1, Study III, Table I.

The animal was given 5 cc. per kilogram of a N/2 solution of hydrochloric. There developed a marked disturbance in the acid-base equilibrium of the blood and a progressive reduction in urine formation. At the termination of the experiment the animal was forming only 2 drops of urine per minute. The reserve alkali at the end of the experiment was 7.9. The elimination of phenolsulphonephthalein was reduced from the normal of 76 per cent. to 34 per cent. Albumin and casts were present in the urine.

At A, is shown a normal glomerulus. At B, are shown convoluted tubules with the epithelium severely swollen. Vacuolation and necrosis are occasionally seen.

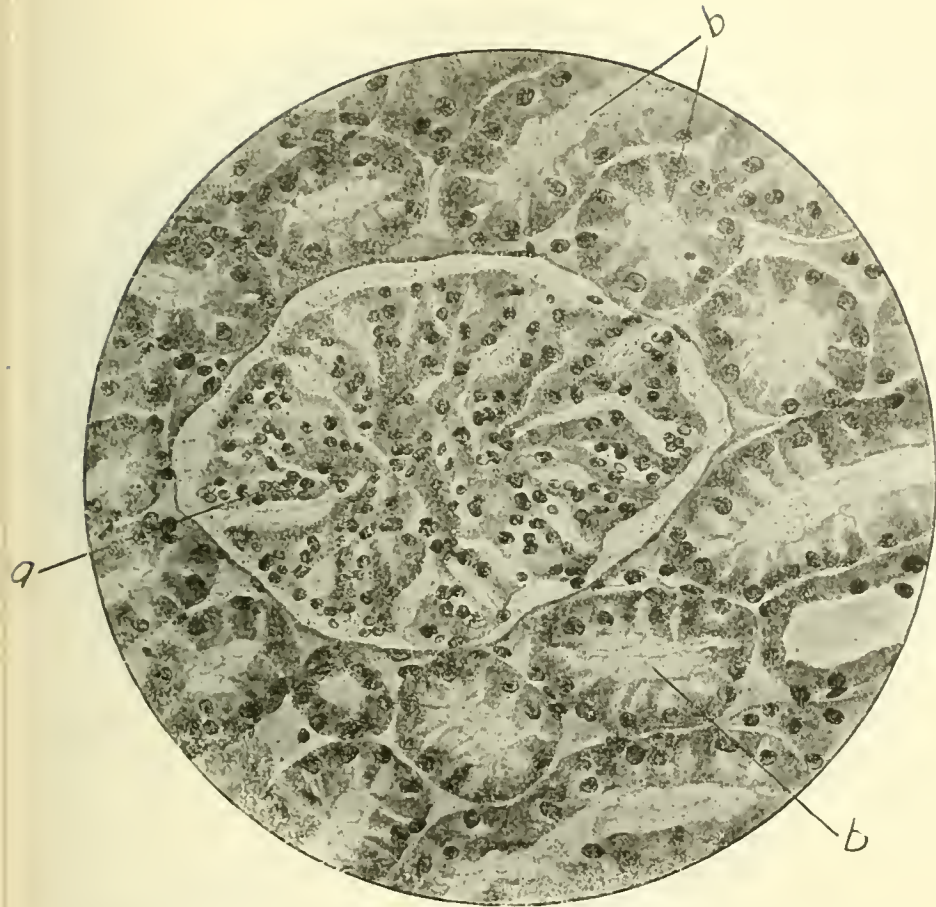


Fig. 2. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the normal animal of Experiment 5, Study III, Table I.

The animal was protected against the toxic effect of a N/2 solution of hydrochloric acid by a preliminary intravenous injection of 25 cc. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride. The acid-base equilibrium of the blood was well maintained throughout the experiment. The reserve alkali of the blood at the conclusion of the experiment was 8.05 as opposed to the normal alkali reserve of 8.1. The animal was freely diuretic during the experiment. The flow of urine at the end of the experiment was 12 drops per minute. Neither albumin or casts were present in the urine. The elimination of phenolsulphonephthalein was only reduced from the normal output of 81 per cent. to 65 per cent.

At A, is shown a normal glomerulus which fills the capsular space. At B, are shown convoluted tubules with a shrunken epithelium which stains well. The nuclei are hyperchromatic.



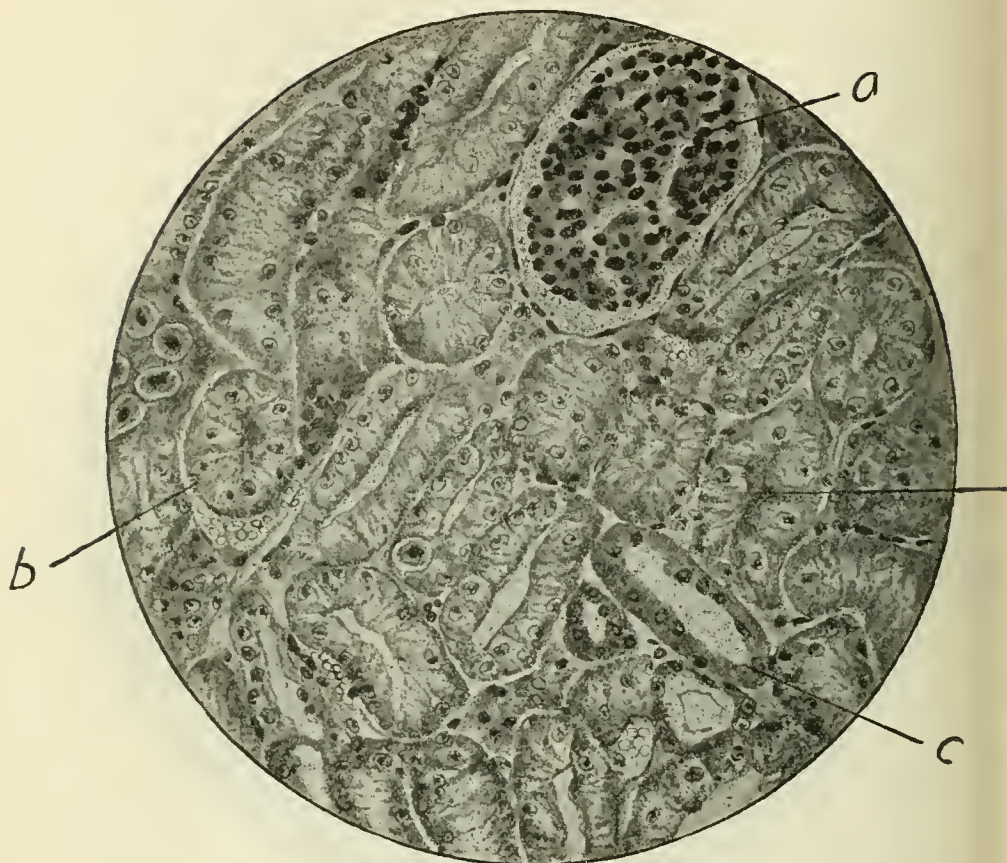


Fig. 3. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the control naturally nephropathic animal of Experiment 1, Study III, Table I.

The animal was given intravenously one injection of 5 cc. per kilogram of a N/2 solution of hydrochloric acid. There developed a marked disturbance in the acid-base equilibrium of the blood and a rapid reduction in urine formation. The reserve alkali of the blood at the termination of the experiment was 7.75 as opposed to the normal reading of 8.0. At the end of the experiment the animal was anuric. During the experiment the elimination of phenolsulphonaphthalein was reduced from the normal of 52 per cent. to a trace.

At A, is shown a glomerulus with an increase in endothelial nuclei and a matting together of the capillary loops. At B, are shown convoluted tubules in an advanced stage of edema and vacuolation. Necrosis of the epithelium is well marked. At C, are shown collecting tubules in which these degenerative changes are less pronounced.

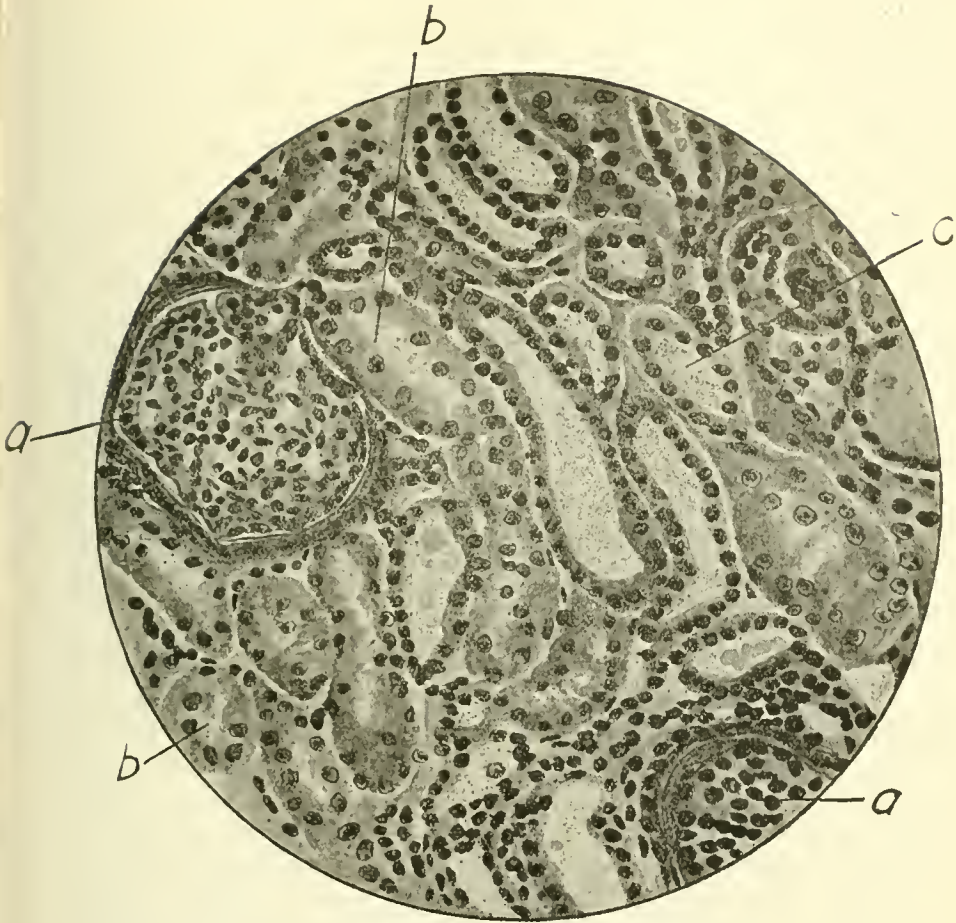


Fig. 4. Camera lucida drawing. Leitz Oc. 2, obj. 6.

The figure is from the unprotected naturally nephropathic animal of Experiment 9, Study III, Table I.

The animal was in part protected against the toxic effect of a N/2 solution of hydrochloric acid by a preliminary intravenous injection of 25 cc. per kilogram of a solution of sodium carbonate equimolecular with a 1.5 per cent. solution of sodium chloride. The normal acid-base equilibrium of the blood was imperfectly maintained during the experiment. At the end of the experiment the reserve alkali was 8.0 as opposed to the normal reading of 8.05. The animal was diuretic throughout the experiment and at its termination was forming 8 drops of urine per minute. The urine contained a trace of albumin but no casts. The elimination of phenolsulphonaphthalein was reduced from the normal of 38 per cent. to 22 per cent.

At A, are shown glomeruli in an early stage of an intracapillary fibrosis. The capsules of the glomeruli are thickened. At B, are shown convoluted tubules in which there is an early edema. Vacuolation and necrosis of the epithelium is rarely seen. At C, are shown the tubules of the loops of Henle which show to a less extent these changes of degeneration.

TABLE 11. TABLE 1.

THE ABILITY OF AN ALKALINE SOLUTION TO FACTOR THE KIDNEY OF NORMAL AND RARELY ENOPATHIC DOGS ADJUSTS TO ACID SOLUTION.

[illegible]



## STUDIES OF THE VITAMINE POTENCY OF COD LIVER OILS.

### IV.—TO WHAT EXTENT IS QUANTITATIVE ESTI- MATION OF VITAMINE A POSSIBLE?

BY ARTHUR D. HOLMES.

*Research Laboratories, E. L. Patch Company, Boston, Mass.*

The investigators who have been collecting information concerning the source and the nature of vitamins have given no small amount of attention to the qualitative phase of the subject, and as a consequence much information is available regarding the vitamins that are present in materials used for nutritive or therapeutic purposes. On the other hand few investigators have made a quantitative study of the vitamin present in materials under consideration. This situation is natural for in the development of a subject of this nature it is desirable to first find out what materials contain vitamins, but valuable and necessary as such knowledge is, it is not sufficient for all purposes. In cases of limited or abnormal dietaries it is often important to have some quantitative information concerning the vitamin content of a small list of foods which may be included in the diet of an invalid, convalescent, or small child. Though such data may be important for the dietitian it is even more necessary that the physician have definite data concerning the vitamin content of the materials which he proposes to employ for vitamin therapy, just the same as he requires data concerning the potency of thyroid, digitalis, ergot and similar medicinal preparations.

Cod liver oil is the best known, most widely used and the richest source of vitamin A employed for therapeutic purposes. However, in spite of this fact there is a decided paucity of data relative to the actual vitamin A potency of commercial cod liver oils. From a review of the literature and interviews with pediatricians it appears that in the therapeutic use of cod liver oil it is quite often administered in amounts which are decidedly in excess of the vitamin A requirements of the patient. Considered from a dietary standpoint only, the practice of administering large doses of cod liver oil is often desirable, for it has a high energy value (nearly 4100 calories per pound) and, as reported in an earlier paper<sup>1</sup> human digestion experiments show

it to be approximately 98% digested. From these facts it is evident that the administration of large doses of cod liver oil may often be recommended in the case of patients who tolerate fats well. But for patients with a low fat tolerance large doses of cod liver oil may serve only to eliminate the possible beneficial results which this medicinal agent might produce. Especially is this true of infants that have a low tolerance for fats, and it is for such patients that the pediatrician requires a cod liver oil of high vitamine potency.

In planning for an investigation which was designed to accumulate data concerning the vitamine A potency of commercial cod liver oils, it was early recognized that it was essential to secure quantitative data, for otherwise it would be impossible to determine the influence of many factors such as the condition of the cod livers, the manufacturing processes and the storage of the cod liver oil, on the vitamine A potency of the final product. As has been noted elsewhere<sup>2</sup> it required only a brief survey of the cod liver oil industry to show that the cod liver oil of commerce is often not from cod livers only, but instead it is a mixture of fish liver oils in which cod liver oil predominates. Consequently in order to ascertain the vitamine potency of combinations of various liver oils our investigations of a necessity include a study of many fish liver oils.

Some investigators in reporting the results of their studies of the vitamine A potency of cod liver oils have referred to dairy butter as a standard for comparison. Since it has been shown that the vitamine A content of dairy butter varies with the kind of ration that the cow consumes it is apparent that unstandardized dairy butter is not entirely satisfactory as a standard of vitamine A. It therefore seems that in determining the vitamine A potency of oils it is preferable to determine what amount of the oil in question is required to produce definite growth, and from studies of this character it has been found that it is possible to determine within narrow limits the quantity of cod liver oil necessary to provide an adequate amount of vitamine A to meet the body requirements of experimental animals. In view of the success that has been attained in this direction it seems desirable

1. *U. S. Dept. Agri. Bul.* 1033, July, 1922, p. 3.

2. *Journ. Metabolic Research*, Vol. II, No. 3, Sept., 1922, p. 361.

for the interest of other investigators, to report briefly concerning our laboratory procedure and some typical results that were obtained for a commercial cod liver oil that was recently tested for its vitamine A potency.

The albino rat has been employed as a laboratory animal because its food habits are very similar to those of man, it requires a small amount of space and food for maintenance and its life cycle is short, so that data concerning growth and maintenance can be secured within a short period of time.

In these tests young growing rats of approximately 40 gm. weight were selected. They were fed an experimental vitamine-free diet consisting of casein 18%, peanut oil 22%, corn starch 28%, milk sugar 28% and salt mixture 4%. This diet was made adequate as regards vitamine B by feeding each animal 0.2 gm. of dried brewer's yeast daily.

The accompanying chart supplies detailed data concerning changes in body weight and food intake of the experimental animals during the vitamine tests. It will be noted from the growth curves that all the animals grew at a very satisfactory rate until they had exhausted their body reserve of vitamine A, after which they began to decline in weight rather rapidly. When unmistakable symptoms of malnutrition and xerophthamia were evident, the diet of the experimental animals was supplemented by cod liver oil in amounts varying from 0.00025 gm. to 0.005 gm. daily. Check tests were made for the 0.25 mg., 0.5, and 0.75, and 1.0 mg. tests, but the results with the 2.0, 3.0, 4.0 and 5.0 mg. tests were so positive that check tests were not made for these amounts of cod liver oil. Data concerning the food intake of the experimental animals are supplied by food curves which report the amount of the experimental diet consumed during successive five day periods.

It is of more than passing interest to note the sharp line of demarkation between the amounts of this oil which will and which will not suffice for good growth of the experimental animal. All the animals that received 0.75 mg. or less of oil daily, died, while all the animals that received 1.0 mg. or more of oil daily made rapid recovery from pronounced malnutrition. As yet it is not known at what point between 0.75 and 1.0 mg. the experimental

animals could just be maintained with this particular cod liver oil. Tests have been made with a number of cod liver oils of both domestic and foreign manufacture to ascertain whether it is possible by this type of analytical procedure to determine the vitamine A potency of average commercial cod liver oils. The results of these tests of cod liver oils were of the same character as those reported above, but the amount of cod liver oil required to produce good growth varied with different oils, indicating quite conclusively that the vitamine A potency of commercial cod liver oils is far from uniform. One or two domestic cod liver oils tested showed a materially higher vitamine A potency than the oil discussed above, and on the other hand two lots of foreign cod liver oils had so much lower vitamine A potency that sixteen mg. daily were not sufficient to keep the experimental animals alive. Such results show that it is possible by feeding graduated doses of cod liver or other edible oils to standardized animals under standardized laboratory conditions to determine within very narrow limits the amount of oil required daily to supply an adequate amount of vitamine A to produce satisfactory growth in experimental animals.

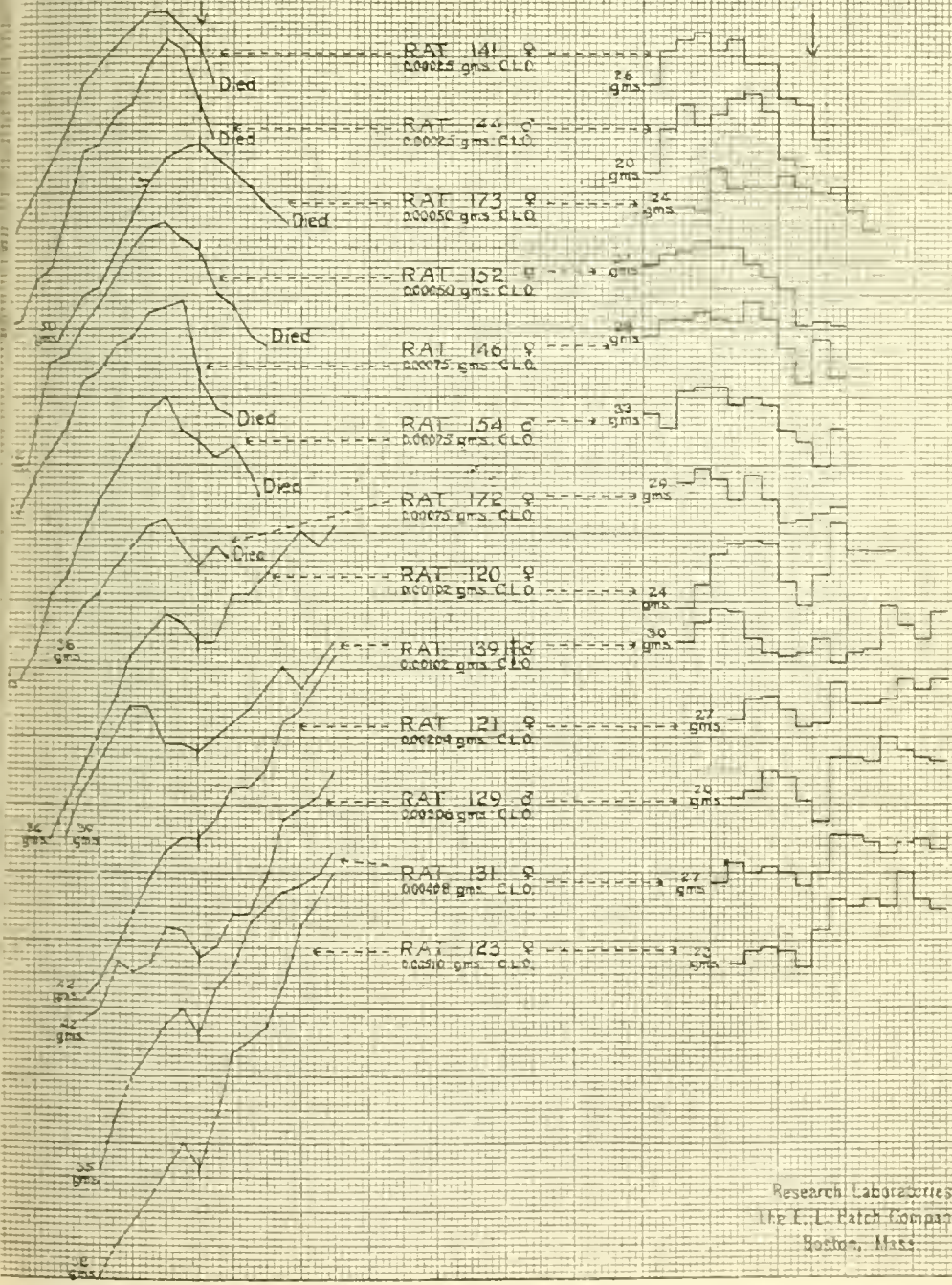
By means of the analytical procedure outlined above, one does not obtain quantitative data concerning the vitamine A content of a given oil, but by strictly adhering to this analytical procedure one may easily determine the relative vitamine A potency of edible fats and oils. As evidence of the importance of information concerning the relative vitamine A potency of cod liver oils, it is only necessary to note that we have found the vitamine A potency of one sample of commercial cod liver oil to be one hundred times that of a second sample of cod liver oil obtained from the open market. Such data justify the present day pediatrician's demand for definite data concerning the vitamine potency of cod liver oil that he purposes to use for vitamine therapy.



# VITAMIN POTENCY OF MEDICINAL COD LIVER OIL Chart 10

Started Cod Liver Oil

Started Cod Liver Oil



Research Laboratories  
The E. L. Patch Company,  
Boston, Mass.





## EXPERIMENTAL STUDIES IN DIABETES.

Series II. The Internal Pancreatic Function in Relation to Body Mass and Metabolism.

### 11. The Relation of the Adrenals to Diabetes.

BY FREDERICK M. ALLEN, M.D.

*From the Hospital of the Rockefeller Institute for Medical Research, New York.*

In continuance of an earlier investigation,<sup>1</sup> the writer performed experiments in the years 1917 and 1918 concerning possible relationships between the pancreas and adrenals in regard to the etiology or symptoms of diabetes. The three lines of experiment consisted in partial epinephrectomy, fat feeding in connection with epinephrin injections, and epinephrin injections in partially depancreatized dogs.

#### 1. *Partial Epinephrectomy.*

The low blood sugar of patients with Addison's disease and the still more marked hypoglycemia of totally epinephrectomized animals were observed by authors previously quoted<sup>1, 2, 3</sup> and confirmed by practically all later workers.<sup>4, 5, 6, 7</sup> It was also demonstrated that the hyperglycemia and glycosuria ordinarily following pancreatectomy are completely prevented when both adrenals are removed at or near the same time<sup>8, 9, 10</sup> (but not when they are removed at the height of the diabetes<sup>11, 12</sup>). Such observations furnished the chief support of the pluriglandular doctrine of diabetes. Subsequent work, however, has proved (a) that the conditions in adrenal deficiency in many respects resemble any other state of collapse,<sup>13, 14, 15</sup> (b) that the early death following bilateral epinephrectomy, as also the hypoglycemia and other symptoms, are due to loss of the cortical and not the medullary portion of the adrenals, because animals survive if they possess sufficient accessory cortical substance or if a sufficient portion of the cortex be spared,<sup>1, 16, 17, 18</sup> (c) that carbohydrate metabolism is unaffected and diabetic and other forms of glycosuria occur as usual after operations which thus leave sufficient cortical tissue while removing the entire chromaffin tissue of the adrenals.<sup>19, 19, 20, 21</sup> This evidence was less complete at the time when the experiments here to be presented were performed, but as the method was different from that used by others, the results may still be worth recording.

A series of experiments were performed by the removal of as much adrenal tissue as seemed compatible with life, and testing the possible influence upon carbohydrate metabolism. Intravenous glucose injections were chiefly employed, by the discontinuous method previously described,<sup>22</sup> to guard against any irregularities of absorption under these conditions. The other methods were the same as in former papers.

Dogs F6-83, F6-84 and G7-39 were normal animals which underwent removal of the greater part of the left adrenal and subsequently of the entire right adrenal, leaving only about one-sixth of the total adrenal tissue, and which received intravenous glucose tolerance tests a few days before the first operation and 2 or 3 weeks after the second operation. The results of these tests, shown in Table 1, indicate certainly no elevation of tolerance by the reduction of adrenal tissue. A lowering of assimilation might be suspected especially in dog F6-84, but all the differences are probably within accidental variations.

TABLE 1.

Dogs receiving intravenous tolerance tests with 1.5 gm. Merck glucose per kg. per hour in 10% solution, 3 injections per hour for 3 hours, before and after partial epinephrectomy.

Dog F6-83 Weight 9 Kg.				Dog F6-84 Weight 9 Kg.			Dog G7-39 Weight 11 Kg.		
	URINE		Plasma Sugar %	URINE		Plasma Sugar %	URINE		Plasma Sugar %
	Vol. cc.	Glucose %		Vol. cc.	Glucose %		Vol. cc.	Glucose %	
<b>Before Operation</b>									
Before injection	25	0	0.109	18	0	0.086	15	0	0.118
End of 1st hour	52	3.70	0.159	50	2.63	0.175	10	2.00	0.167
End of 2nd hour	58	2.86	0.152	26	2.80	0.179	5	2.80	0.137
End of 3rd hour	124	2.83	0.218	90	1.50	0.104	148	0.26	0.232
1 hour after ending injections	38	Faint	0.102	63	Faint	0.075	44	Faint	0.085
2 hours after ending injections	12	Faint	0.114	25	Faint	0.089	66	0	0.131
<b>After Operation</b>									
Before injection	38	0	0.099	10	0	0.094	56	0	0.088
End of 1st hour	41	4.08	0.263	26	8.70	0.303	30	0.87	0.219
End of 2nd hour	40	1.77	0.256	23	4.08	0.244	32	0.88	0.145
End of 3rd hour	71	1.24	0.145	138	3.23	0.179	60	0.41	0.156
1 hour after ending injections	53	0.28	0.089	25	0.26	0.077	86	Faint	0.084
2 hours after ending injections	19	Faint	0.093	20	Faint	0.095	34	0	0.088

## DOG E5-92.

## Partial epinephrectomy in diabetes.

Female, mongrel, age 3 or 4 years, good condition; weight 16.75 kg. August 31, 1917, removal of pancreatic tissue weighing 29.6 gm. Remnant about main duct estimated at 3.4 gm. (1/9—1/10). High carbohydrate

diets at first were necessary for glycosuria, but the diabetes was allowed to progress to a severe stage, so that by repeated tests the tolerance was found to be below 400 gm. of beef lung. Thus, on November 28, the weight was 12.5 kg.; the plasma sugar was 0.167 per cent. before feeding; 6 hours after feeding 400 gm. lung and 100 gm. suet it was 0.269 per cent., and the urine was 395 cc. with 2.4 per cent. sugar. After a fast-day to stop glycosuria,  $\frac{2}{3}$  to  $\frac{3}{4}$  of the left adrenal was removed on Nov. 30. The diet on the next day was 100 gm. lung and 100 gm. suet, and was increased gradually to 400 gm. lung and 100 gm. suet on December 4, when glycosuria returned. It ceased when the lung was reduced to 100 gm., returned with an increase to 300 gm. on December 8, and was continuous on 300 gm. lung and 100 gm. suet to December 12, with plasma sugars as high as 0.4 per cent.

December 12, at a weight of 11.7 kg., the entire right adrenal was removed by an operation which was very easy in the emaciated dog. Glycosuria was stopped by the one fast-day, and failed to return on the diet of 300 gm. lung and 100 gm. suet. A sufficient reason was present in the cachexia and diarrhea of poorly digested food. On December 27 the weight was 11 kg., the plasma sugar 0.147 per cent. before feeding and 0.185 per cent. six hours after feeding. The weight and strength continued to fail, until on January 18, 1918, the dog weighed 9.5 kg. and was too feeble to stand. The plasma sugar was 0.042 per cent., the CO<sub>2</sub> capacity 34.7 vol. per cent.; acetone negative. At autopsy the pancreas remnant weighed 3.9 gm., and the results of the adrenal operations were confirmed. Microscopically, the pancreatic acini were empty but not involuted. Islands were present in fair size and number, but with vacuolation in a few cells. This, and some Armanni changes in the kidneys, indicated that hyperglycemia probably continued up to the terminal collapse. The viscera otherwise were negative.

The cachexia and asthenia of this animal were suggestively like those of Addison's disease. Also as noticed in connection with the thyroid,<sup>23</sup> operations on different organs which separately are well borne sometimes produce fatal cachexia when combined. The suppression of glycosuria and partial reduction of hyperglycemia here are not appreciably different from the rule in any cachexia.<sup>24</sup> No benefit of the adrenal operations is perceptible, for the resulting condition was fatal, while many experiments in this and the preceding series have shown that with simple undernutrition the diabetes in such a case can be controlled and the animal kept alive apparently indefinitely.

## *2. Fat Feeding in Relation to Epinephrin Glycosuria.*

It was asserted by Blum<sup>25</sup> and confirmed by Roubitschek<sup>26</sup> that when animals have fasted to such a point that a certain dose of epinephrin no longer causes glycosuria, the feeding of pure fat for several days causes them to be subject to glycosuria from the same dose. These writers interpreted their results as proof of the

formation of sugar from fat. Eppinger, Falta and Rudinger<sup>27</sup> reported a similar finding, but were inclined to believe that the fat merely spared glycogen. Though these reports are inherently improbable, the experiments seemed worth repeating for two reasons: (a) It is generally accepted that fat tends to drive out glycogen from the liver, and it is thus conceivable that fat feeding might augment the effect of epinephrin; (b) Fat exerts a powerful influence upon diabetic glycosuria,<sup>28, 29</sup> and a similar observation in connection with any other form of glycosuria would be of interest.

Numerous trials were made which need not be reported in detail, as they merely confirm Underhill's<sup>29, 30</sup> conclusion that this form of experiment is unreliable; no uniformity of either hyperglycemia or glycosuria can be anticipated in the same animal or in different animals from the same dose of epinephrin. The influence of single or repeated fat feedings upon the sugar of blood or urine was negative throughout. This statement holds not only for normal dogs but also for those depancreatized so as to lower the tolerance nearly to the point of diabetes. A single experiment of this type will be given as an illustration.

Dog B2-00, normally weighing 14 kg., had been subjected to partial pancreatectomy to such an extent that the removal of approximately 2.5 gm. of tissue was subsequently found necessary to produce diabetes. Fasting was begun May 8, 1916, at a weight of 14.3 kg. May 15, at a weight of 12.9 kg., a subcutaneous injection of 4 cc. of Parke-Davis adrenalin solution (1/1000) was given in the fasting condition, with the result of hyperglycemia without glycosuria, as shown in Table 2.

TABLE 2

Dog B2-00.

Weight 14 kg. Partially depancreatized, not quite diabetic.  
Injection of 4 cc. adrenalin solution (1/1000) subcutaneously at 2:45 P. M.  
on 4 different days.

Time	PLASMA SUGAR %				Remarks
	May 15	May 22	May 26	May 29	
2:40 P. M.	0.123	0.095	0.105	0.092	May 15, fasting.
3:45 P. M.	0.126	0.110	0.104	.....	" 22, fed 200 gm. lard.
4:45 P. M.	0.175	0.095	0.137	0.154	" 26, fed 300 gm. white clay.
6:00 P. M.	0.196	0.161	0.162	0.228	" 29, fed 500 gm. lung.
7:30 P. M.	0.200	0.186	0.250	0.250	.....
10:30 P. M.	0.170	0.230	0.244	0.323	.....
Total glucose excreted, gm.	0	0	0	0.12	.....



May 17, the feeding of 100 gm. lard daily was begun. May 22, at a weight of 11.9 kg., 200 gm. lard was fed at 9 A. M., and at 2:45 P. M. an epinephrin injection given as before. There was still no glycosuria, and the difference in hyperglycemia was within the limits of accidental variation.

Plain fasting was then resumed. May 26, 300 gm. white clay was fed at 9 A. M., by mixing with water and molding in convenient lumps, each bolus being swallowed readily when placed in the back of the mouth. The purpose was to test any possible influence of mere fullness of the stomach. There was still no glycosuria from the adrenalin injected in the afternoon, and no important difference in the hyperglycemia.

With continuance of fasting to May 29, the weight was down to 10.9 kg., and the animal was distinctly weak. After the feeding of 500 gm. of beef lung, the same dose of adrenalin produced the highest hyperglycemia of the series, together with slight glycosuria. Though there were fewer calories and fewer grams of protein on this day than of fat on May 22, and though it was at a later stage of fasting, the sugar production from protein was nevertheless positive, in contrast to the negative results of fat.

### *3. Epinephrin Injections in Relation to Diabetes.*

The experiments under this heading were planned from three points of view.

First, diabetic dogs are subject to hydropic degeneration of the islands of Langerhans from the functional overstrain of excessive diets.<sup>31</sup> If epinephrin is specifically antagonistic to the island function, it may perhaps produce morphologic changes in animals predisposed by suitable partial pancreatectomy.

Second, if, as alleged in pluriglandular speculations, the hormone of the islands normally acts as a "brake" upon sugar formation in the liver, and diabetes consists in excessive glycogenolysis by epinephrin due to the removal of this "brake," exaggerated effects should be produced by epinephrin injections in animals made diabetic by partial pancreatectomy.

Third, diabetes is characterized by excessive and persistent formation of sugar not merely from glycogen but also from protein. Several authors<sup>32</sup> have described increased excretion of nitrogen in fasting animals as a result of epinephrin injections. Differences of dosage and methods may explain various discrepancies. The most reasonable explanation is that after depletion of its glycogen by epinephrin the fasting organism undertakes to

restore its glycogen reserve, and does so by using protein for the purpose. In this way the increased nitrogen excretion is merely a secondary phenomenon, and the general experience is that epinephrin under ordinary conditions of nutrition does not increase nitrogen loss. Observations on this subject in partially depancreatized dogs seemed desirable for two reasons: (a) to determine whether under these circumstances epinephrin will reproduce this important feature of diabetes, namely, glycosuria at the expense of increased protein destruction; (b) in addition for possible light upon a new question, namely, whether diabetic tissues are more easily subject to breakdown from various causes than the normal.

For this purpose, a series of diabetic dogs and normal controls were subjected to experiments as follows:

#### DOGS D4-77 and D4-89.

Dog D4-77 was partially depancreatized January 19, 1917, leaving a remnant estimated at about one-ninth about the main duct. The mild diabetes which resulted was made severe by carbohydrate overfeeding, and the condition was then kept under control by protein-fat diet and undernutrition. Dog D4-89 was chosen as a normal control and subjected to the same conditions, except operation. The two dogs were then used for experiments with thyroid feeding, as previously described.<sup>22</sup> Following the thyroid period, injections of adrenalin were given as shown in the tables, first on the diet mentioned and later on fasting.

Though dog D4-77 actually had diabetes of considerable severity, and was only kept sugar-free by close dietary restriction, no marked exaggeration of the effects of either intravenous or subcutaneous adrenalin doses was demonstrable. There was no glycosuria in either animal, and the differences in regard to hyperglycemia were not extreme. The nitrogen loss of the two dogs was also practically identical.

#### DOGS E5-00 and E5-46.

Dog E5-00 was made severely diabetic by successive pancreatic operations on March 8 and April 3, 1917, the remnant about the main duct being estimated at between one-ninth and one-tenth of the gland. After the glycosuria had become well established, it was checked by fasting and low protein-fat diet. Dog E5-46 was kept on the same diet, as a normal control.

The severity of the diabetes in dog E5-00 is indicated by the glycosuria which resulted from the feeding of only 200 gm. beef lung and 100 gm. suet on May 29. A fast day, followed by a day of suet only, raised the tolerance so that this quantity of protein was tolerated following June 1. The subsequent intraperitoneal adrenalin injections produced no glycosuria in the fasting normal dog and only trivial sugar excretion in the severely diabetic dog. The nitrogen excretion per kilogram happened to be identical in the two animals.





TABLE 5.  
Dog E5-00.

Date 1917	Time	BLOOD		URINE				Body Weight kg.	Remarks
		Plasma Sugar mg. per 100 cc.	CO <sub>2</sub> vol. %	Vol. cc.	Sugar gm.	Acetone qual.	Total-N gm.		
May 29		.....	.....	463	4.00	Neg.	5.65	14.5	Fed 300 gm. lung, 100 gm. suet.
" 30		.....	.....	390	Faint	"	4.34	.....	Not fed.
" 31		.....	.....	368	Neg.	"	7.56	.....	Fed 200 gm. suet.
June 1		.....	.....	328	"	"	3.44	.....	Fed 300 gm. lung, 100 gm. suet.
" 2		.....	.....	456	"	"	5.70	.....	" " 200 gm. "
" 3		.....	.....	432	"	"	5.75	.....	" " "
" 4		.....	.....	810	"	"	8.04	.....	Not fed.
" 5		.....	.....	512	"	"	3.24	.....	Fed 300 gm. lung, 200 gm. suet.
" 6		.....	.....	470	"	"	5.85	.....	" " "
" 7	6:00 P. M.	101	.....	536	"	"	4.94	.....	Not fed. Injected 2 cc. adrenalin intraperi-
" 8	9:00 P. M.	196	.....	445	Faint	"	4.14	.....	toneally.
" 9	10:30 A. M.	147	.....	.....	.....	.....	.....	.....	Not fed. Injected 4 cc. adrenalin intraperi-
" 10	3:00 P. M.	346	.....	.....	.....	.....	.....	.....	toneally at 12:30 P. M.
" 11	10:00 P. M.	159	.....	208	2.20	"	3.30	13.3	Not fed. Injected 6 cc. adrenalin.
" 12	9:15 A. M.	161	.....	.....	.....	.....	.....	.....	.....
" 13	12:00 M.	344	.....	.....	.....	.....	.....	.....	.....
" 14	1:30 P. M.	90	.....	348	Neg.	"	4.16	.....	No adrenalin today.
" 15	6:00 P. M.	263	50.4	200	Faint	"	2.34	.....	Not fed. Injected 8 cc. adrenalin intraperi-
" 16	9:30 A. M.	112	57.9	275	Neg.	"	2.41	.....	toneally.
" 17	11:00 A. M.	90	.....	270	"	"	2.21	.....	No adrenalin today.
" 18	8:00 P. M.	222	.....	432	"	"	4.08	.....	.....

Total N output for 17 days

Average N output per day

N output per kg. per day

77.15

4.54

.31



TABLE 6.  
Dog E5-46.

Date 1917	Time	BLOOD		URINE				Body Weight kg.	Remarks
		Plasma Sugar mg. per 100 cc.	CO <sub>2</sub> vol. %	Vol. cc.	Sugar gm.	Acetone qual.	Total-N gm.		
May 29		---	---	199	Neg.	Neg.	6.68	15.5	Diet 300 gm. lung, 100 gm. suet.
" 30		---	---	210	"	"	7.90	---	Not fed.
" 31		---	---	128	"	"	---	15.35	Diet 200 gm. suet.
June 1		---	---	118	"	"	3.68	15.	Fed 300 gm. lung, 100 gm. suet.
" 2		---	---	145	"	"	4.72	15.25	" " 200 gm. "
" 3		---	---	No	Urine	---	---	---	---
" 4		---	---	480	Neg.	Neg.	18.30	15.6	Not fed.
" 5		---	---	108	"	"	3.16	15.25	Fed 300 gm. lung, 200 gm. suet.
" 6		---	---	160	"	"	3.14	15.35	" " "
" 7	6:00 P. M.	65	---	342	"	"	7.44	15.55	Not fed. Injected 6 cc. adrenalin in peritoneum.
" 8	9:00 P. M.	164	---	162	"	"	4.78	---	Not fed. Injected 4 cc. adrenalin in peritoneum.
" 10:30 A. M.		103	---	---	---	---	---	---	---
" 3:00 P. M.		151	---	---	---	---	---	---	---
" 10:00 P. M.		85	---	238	"	"	5.83	---	Not fed. Injected 6 cc. adrenalin in peritoneum.
" 9:15 A. M.		62	---	---	"	"	---	---	---
" 12:00 M.		156	---	---	"	"	---	---	---
" 10		---	---	190	"	"	5.18	---	No adrenalin today. Not fed.
" 11	1:30 P. M.	88	44.7	68	"	"	1.82	---	Not fed. Injected 8 cc. adrenalin intraperitoneally.
" 12	9:30 A. M.	54	41.9	100	"	"	1.25	13.	No adrenalin today.

73.88

Total N output for 15 days

4.92

Average N output per day

.31

N output per kg. per day

TABLE 7.  
Dog, F6-07.

Date 1917		BLOOD			URINE					Body Weight kg.	Remarks	
		Plasma Sugar mg. per 100 cc.	CO <sub>2</sub> vol. %	Corp. vol. %	Vol. cc.	Sugar gm.	Acetone qual.	Total-N gm.	NH <sub>4</sub> -N gm.			
Dec.	8	11:00	122	.....	.....	600	4.40	Neg.	8.88	0.33	14.5	Injected 6 cc. adrenalin into the peritoneum.
"	9	1:00	345	.....	.....	360	1.62	"	5.16	0.24	.....	Injected 6 cc. adrenalin.
"	10	11:45	172	70.0	48.5	300	Faint	"	3.66	0.16	.....	" 8 cc.
		2:00	833	65.3	50.0	.....	.....	.....	.....	.....	.....	.....
		5:00	192	59.5	47.2	.....	.....	.....	.....	.....	.....	.....
"	11	.....	.....	.....	.....	400	Neg.	"	5.36	0.27	.....	Injected 6 cc. adrenalin.
"	12	.....	.....	.....	.....	220	"	"	3.64	0.19	.....	" "
"	13	12:00	143	50.0	45.6	500	Faint	"	3.05	0.18	.....	" "
		2:00	322	59.5	43.3	.....	.....	.....	.....	.....	.....	.....
		4:45	232	66.2	46.5	.....	.....	.....	.....	.....	.....	.....
"	14	.....	.....	.....	.....	360	Neg.	"	5.52	0.30	.....	No adrenalin. Hind legs spastic.
"	15	.....	.....	.....	.....	200	Mod.	"	5.26	0.26	.....	Injected 8 cc. adrenalin.
"	16	.....	178	63.3	42.3	145	Neg.	"	3.84	0.12	.....	.....
Total N output for 9 days									44.37			
Average N output per day									4.93			
N output per kg. per day									.34			

TABLE 8.  
Dog F6-08.

Date 1917	Time	BLOOD			URINE					Body Weight kg.	Remarks
		Plasma Sugar mg. per 100 cc.	CO <sub>2</sub> vol. %	Corp. vol. %	Vol. cc.	Sugar gm.	Acetone qual.	Total-N gm.	NH <sub>3</sub> -N gm.		
Dec. 8	11:00 A. M.	179	.....	.....	640	Neg.	Neg.	10.36	0.59	15.0	.....
" 9	"	.....	.....	.....	240	"	"	3.87	0.20	.....	.....
" 10	2:00 P. M.	151	.....	41.0	400	"	"	4.88	0.32	.....	.....
" 11	"	.....	64.3	.....	240	"	"	3.48	0.07	.....	.....
" 12	"	.....	.....	.....	250	"	"	3.84	0.19	.....	.....
" 13	5:15 P. M.	93	.....	41.2	300	"	"	5.46	0.30	.....	.....
" 14	"	.....	62.4	.....	200	"	"	4.84	0.16	12.2	0.5 gm. pancreatic tissue re- moved.
" 15	"	.....	.....	.....	220	"	"	3.30	0.28	.....	.....
" 16	"	.....	.....	.....	235	"	"	3.53	0.35	.....	.....
" 17	"	.....	.....	.....	240	"	"	4.32	0.28	11.6	.....
" 18	10:30 A. M.	105	.....	61.4	280	Faint	"	4.17	0.18	.....	.....
" 19	"	.....	.....	.....	250	"	"	4.05	0.22	11.0	1.25 gm. pancreatic tissue re- moved.
" 20	"	.....	.....	.....	300	Neg.	"	3.72	0.33	.....	.....
" 21	"	.....	.....	.....	250	"	"	4.65	0.36	.....	.....
" 22	12:45 P. M.	100	.....	.....	280	"	"	3.90	0.33	.....	.....
" 23	"	.....	.....	.....	100	"	"	1.24	0.26	.....	.....
" 24	12:30 P. M.	130	.....	31.9	200	"	"	4.10	0.19	.....	Fed 100 gm. bacon grease.
" 25	"	.....	52.8	.....	120	"	"	1.82	0.13	.....	" " "
" 26	"	.....	.....	.....	170	"	"	1.78	0.17	.....	" " "
" 27	"	.....	.....	.....	230	"	"	3.63	.....	.....	" 50 gm. bacon grease and 50 gm. lard.
" 28	"	.....	.....	.....	150	"	"	1.82	0.40	.....	.....
" 29	"	.....	.....	.....	130	"	"	1.36	0.12	9.1	.....
" 30	"	.....	.....	.....	.....	.....	.....	.....	.....	.....	This 24 hr. urine lost.
" 31	5:00 P. M.	256	53.8	31.1	180	Neg.	Neg.	2.22	0.17	8.5	.....

Total N output for 24 days.  
Average N output per day.  
N output per kg. per day.

86.34  
3.59  
.24

## DOGS F6-07 and F6-08.

These two animals were chosen as nearly equal in weight, and were partially depancreatized on December 6, 1917. The remnant communicating with the main duct was estimated in dog F6-07 as one-eleventh, and in dog F6-08 as one-twenty-first of the gland; i.e., under ordinary circumstances the diabetes would be more severe in the latter dog. By reason of fasting one day before operation and continuously afterwards, dog F6-08 remained free from glycosuria. There was rapid enlargement of the pancreas remnant, the weight of which was estimated at 1.4 gm. on December 6. On December 14, 0.5 gm. additional was removed, and on December 19 an additional 1.25 gm., and when the dog was chloroformed for autopsy on December 31 the remnant still weighed 1.9 gm. The pancreas specimens from all the operations and the autopsy were microscopically normal, except for doubtful thinning of cytoplasm in the island cells at the close, as though a slight tendency to exhaustion might be present.

The pancreas remnant in dog F6-07 was estimated at 2.5 gm., and at autopsy on December 16 was found to weigh 2.6 gm. The difference in hypertrophy as compared with dog F6-08 need not be attributed to adrenalin, as different dogs vary widely in this respect for unknown causes.<sup>21</sup> The pancreatic tissue at autopsy was microscopically normal except for slight but distinct vacuolation in a number of cells of all the islands.

Slight glycosuria resulted from the intraperitoneal adrenalin injections in dog F6-07. This effect was less than might have been anticipated from the severity of the potential diabetes, and the progressiveness characteristic of diabetes seemed to be absent. Excessive protein catabolism was also absent.

Though the familiar transitory adrenalin glycosuria is represented here, no definite influence toward the production of true diabetes is demonstrable, though mere over-feeding would have shown this effect very quickly. The rapid hypertrophy of the pancreas remnant in dog F6-08 may have more than compensated for its smaller size, and the slight difference in vacuolation of islands probably lies within accidental variations. Neither the glycosuria nor the azoturia of true diabetes was in evidence.

TABLE 9.  
Dog F6-06.

Date 1917	Time	BLOOD			URINE				Body Weight kg.	Remarks
		Plasma Sugar mg. per 100 cc.	CO <sub>2</sub> vol. %	Corp. vol. %	Vol. cc.	Sugar gm.	Acetone qual.	Total-N gm.		
Dec. 8	11:00 A. M.	333	.....	.....	800	25.6	Neg.	12.48	19.0	Injected 6 cc. adrenalin intraperitoneally.
" 9	1:00 P. M.	526	.....	.....	1500	55.5	"	9.40	.....	Adrenalin the same as yesterday.
" 10	11:45 A. M.	455	73.9	52.1	1350	44.1	"	14.14	.....	Injected 8 cc. adrenalin in peritoneum.
" 11	2:00 P. M.	435	63.3	49.4	.....	.....	.....	.....	.....	.....
" 12	5:00 P. M.	346	.....	48.2	900	Heavy	"	13.32	.....	.....
" 13	12:00 M.	500	.....	.....	760	"	Slight	9.76	.....	.....
" 14	2:10 P. M.	625	57.6	51.5	900	28.71	Neg.	16.29	.....	.....
" 15	4:45 P. M.	500	77.4	58.0	.....	.....	.....	.....	.....	.....
" 16	.....	.....	.....	.....	800	19.44	"	9.36	.....	0.3 gm. pancreatic tissue removed.
" 17	.....	.....	.....	.....	1000	28.3	"	11.84	.....	Injected 8cc. adrenalin.
" 18	10:30 A. M.	625	.....	.....	1400	32.2	V. faint	15.96	.....	.....
" 19	5:15 P. M.	625	73.9	.....	900	25.2	"	10.53	11.95	.....
" 20	2:00 P. M.	525	60.3	.....	1000	30.9	Neg.	13.76	.....	.....
Total N output for 13 days		.....	55.7	42.2	600	10.8	"	7.68	10.5	0.15 gm. pancreatic tissue removed.
Average N output per day		.....	.....	.....	640	13.72	"	6.72	.....	Autopsy blood.
N output per kg. per day		.....	.....	.....	.....	.....	.....	.....	.....	.....
Total sugar output for 13 days		.....	.....	.....	.....	314.47	.....	.....	.....	.....
Average sugar output per day		.....	.....	.....	.....	24.19	.....	.....	.....	.....
Sugar output per kg. per day		.....	.....	.....	.....	1.27	.....	.....	.....	.....







TABLE 12.  
Dog 1:6-11.

[illegible]

## DOGS F6-06, F6-09, F6-10 and F6-11.

These four animals were chosen as practically identical in size. All of them fasted completely from December 5 onward. On December 6, dog F6-06 was partially depancreatized, leaving a remnant about the main duct estimated at one-seventeenth, and dog F6-09 likewise, leaving a remnant estimated at one-fifteenth of the gland. Dog F6-06 was treated with adrenalin intraperitoneally, while dog F6-09 was used as a diabetic control. Dogs F6-10 and F6-11 were used as non-diabetic controls. The former received adrenalin intraperitoneally in the same dosage as dog F6-06. Dog F6-11 remained on plain fasting until December 19, when partial pancreatectomy was performed, leaving a remnant estimated at one-fifteenth, in order to compare the periods before and after this operation in the same animal. The initial body weight was used as a basis for reckoning the average nitrogen excretion per kilogram.

The output of sugar and nitrogen in dog F6-06 was by far the highest of the series, and the difference seems to lie outside the possible limits of accidental variation. The experiment indicates that in the presence of active diabetes, adrenalin is able to cause a greatly increased excretion of sugar derived not merely from preformed carbohydrate but also from protein.

In the normal dog F6-10, adrenalin caused no glycosuria. The average nitrogen loss was higher than in the first ten-day period of dog F6-11, indicating a possible increase of nitrogen catabolism by adrenalin in a fasting animal, even without loss of sugar. This nitrogen loss, however, was lower than in the diabetic dog F6-09, which received no adrenalin but lost considerable sugar.

The results in dog F6-11 are uncertain. Owing to the prolonged fast, there was very little glycosuria. In figuring the nitrogen loss per kilogram, the initial weight of 19 kg. was taken as a basis for the first period and the weight of 14.55 kg. as a basis for the second period. The average loss thus appears higher during the second period, when the animal was potentially diabetic, but this basis of comparison may not be sufficiently accurate.

The pancreatic tissue of dog F6-06, taken in the original operation on December 6, was normal. The remnant at this operation was estimated at 1.78 gm. After further removal of 0.3 gm. on December 14 and 0.5 gm. on December 19, the remnant at autopsy weighed 2.2 gm. Microscopically, the islands showed vacuolation, increasing from a slight degree on December 14 to a moderate stage at autopsy.

Dog F6-09 had a pancreas remnant estimated at 2.6 gm. on December 6. Additional tissue weighing 0.6 gm. was removed on December 19, and the remnant at autopsy weighed 1.7 gm. The tissue removed December 6 was normal. Distinct vacuolation was present in the islands on December 19, and at autopsy this was fully equal in degree to that found in dog F6-06. The Armani changes in the renal tubules were similar in the two

dogs. No visible changes were therefore produced by the adrenalin doses given to one of these diabetic animals.

Dog F6-10 showed strictly normal pancreatic tissue at autopsy, with no sign of change due to the adrenalin injections. The kidneys showed congestion of glomeruli and slight degeneration and exudation in occasional glomeruli and tubules, which may or may not have been the result of the adrenalin. Armanni vacuolation was slight or uncertain, and the examination was not checked by glycogen or fat stains.

Dog F6-11 showed normal pancreatic tissue in the first operation on December 19, except for partial emptiness of the acini due to fasting. No alteration of islands due to fasting, and none of the alleged transitions between acinar and island tissue were discoverable. Slight vacuolation was found in the islands on December 27, and a slightly more advanced stage at autopsy on December 31. This vacuolation is evidently due to the degree of functional overstrain represented by the marked hyperglycemia, as glycosuria was absent most of the time.

TABLE 13.  
Dog F6-75. May 13, 1918.

Time	BLOOD			URINE			Remarks
	Plasma Sugar mg. per 100 cc.	CO <sub>2</sub> vol. %	Corp. vol. %	Vol. cc.	Sugar gm.	Acetone qual.	
12:30 P. M.	132	46.2	41.8	-----	-----	-----	10 cc. adrenalin injected.
12:55 P. M.	232	51.9	50.0	-----	-----	-----	
1:45 P. M.	555	46.2	49.0	-----	-----	-----	
3:14 P. M.	558	50.0	50.6	-----	-----	-----	
4:15 P. M.	445	61.4	56.7	-----	-----	-----	
7:30 P. M.	99	54.8	68.5	-----	-----	-----	Urine for experimental period.
9:30 P. M.	112	51.9	68.0	92	3.32	Neg.	

#### DOG F6-75.

This normal animal, weighing 7.4 kg., was given 10 cc. of adrenalin solution intraperitoneally at 12:30 P. M. Great hyperglycemia resulted, but the total sugar excretion was only 3.32 gm. The animal was found dying shortly after midnight, and was autopsied immediately. The pancreas was strictly normal, and neither acini nor islands showed the slightest microscopic changes.

### DISCUSSION.

#### *I. Alleged Antagonism of Epinephrin and Insulin.*

With the recent discovery of insulin, the last requirement for complete demonstration of the pancreatic origin of diabetes has been fulfilled. What remains of opposition to this doctrine consists not of facts, but merely a state of mind. The development



of the truth has been so slow that a multitude of persons have become accustomed to pluriglandular conceptions of diabetes, and the most conclusive proof will encounter difficulty in uprooting an error which is so deeply grounded in the literature. There is danger that the same shallow thinking which founded the pluriglandular doctrine will introduce further confusion, by the assumption that epinephrin is the physiological antagonist of insulin. Epinephrin causes glycogenolysis and hyperglycemia; insulin causes glycogen formation and lowering of the blood sugar. What could be a plainer example of antagonism?

As an anatomical example of a similar fallacy, it may be suggested that the chin is lowered when the mouth is opened or the head bent forward, and elevated when a man rises from a sitting posture or leaps into the air; and a person confining his attention exclusively to the chin might thus conclude that the depressors of the mandible are the natural antagonists of the extensors of the legs. If epinephrin is the physiological antagonist of insulin, then the chromaffin tissue is the opponent of the islands of Langerhans, which means that the doctrine of antagonisms and balances between glands is correct, and that diabetes is a form of epinephrin glycosuria, due wholly or largely to the unrestrained action of the adrenals when the inhibiting influence of the Langerhans tissue is weakened. A presentation of experimental evidence on the subject is therefore important at this particular time. Pluriglandism is to be opposed not merely as a false doctrine but especially as an unscientific mode of thought which constitutes the most pernicious tendency in the contemporary study of metabolism. It is therefore worth while to recapitulate briefly some of the known facts bearing on the relation of the adrenals to glycosuria or diabetes, under the following five heads:

1. Clinical Pathology.—It is bad enough that rare abnormalities of the thyroid or hypophysis in association with diabetes have caused these to be dragged into the etiology, but it is well known that for the adrenals no trace of evidence for such a connection exists. The signs of adrenal excess consist in virilism, hirsutism, etc.,<sup>33, 34, 35</sup> and are supposedly due to overdevelopment of the cortical tissue. No state of chromaffin excess has ever been recognized. The numerous attempts to demonstrate abnormal quantities of epinephrin in the blood or in the adrenals have failed

completely in diabetes, hypertension, and all other conditions.<sup>36, 37</sup> Various grades of diabetes and hypertension coexist in numerous elderly patients, doubtless on the basis of associated scleroses in the pancreas and kidneys; but believers in an epinephrin factor have never been able to explain why hypertension is so often found with normal carbohydrate tolerance and why the great mass of diabetics have normal blood pressure.<sup>38</sup> Likewise, states of chromaffin deficiency have never been demonstrated, and there is no evidence that the hypoglycemia of Addison's disease is due to lack of epinephrin.<sup>16</sup>

2. Epinephrectomy Experiments.—It is impossible to learn whether the chromaffin tissue is essential to life, because the greater part of this tissue lies outside of the adrenals and cannot be removed. The cortical tissue, which does not produce epinephrin, is essential to life, and its removal is responsible for the acute death which follows double epinephrectomy in most species. A large proportion of animals of some species, such as the rat and the rabbit, survive this operation because they possess sufficient cortical tissue outside the adrenals.<sup>1, 16, 18</sup> Species such as the cat and dog, in which the double operation is invariably fatal because of lack of sufficient accessory cortical nodules, survive indefinitely when the medullary substance is removed and the cortex spared.<sup>17, 21</sup> Animals which have thus lost the entire adrenal medulla show nothing resembling Addison's disease, have normal blood sugar and carbohydrate metabolism, and are subject to diabetes and various forms of experimental glycosuria in the usual way.<sup>20</sup> The hypoglycemia and other fatal symptoms are due to loss of the cortical tissue, and both the experiments and the interpretations underlying the pluriglandular hypothesis are thus proved to be erroneous.

3. Epinephrin Injections.—The first step in an attempt to show a relationship of the chromaffin tissue to diabetes should be a proof that epinephrin can cause glycosuria. As previously pointed out,<sup>39</sup> such proof has never been brought. The most obvious and characteristic effect of epinephrin in the living organism is the elevation of blood pressure. It remains a fact that the method of administering epinephrin which produces this typical effect to the highest degree, namely intravenous injection, has the least influence upon carbohydrate metabolism, and, *vice versa*, subcutaneous or intraperitoneal injections which cause

little or no rise of blood pressure produce the greatest hyperglycemia and glycosuria. Attempted explanations of this discrepancy are unsatisfactory, and it is a permissible hypothesis that the excess sugar formation is due to chemical products from epinephrin decomposition or from the injured cells, such secondary changes occurring to some extent when the epinephrin is given intravenously but to a greater extent when it is injected directly into the tissues. Epinephrin is supposed, particularly by the pluriglandists, to exert its glycogenolytic influence chiefly in the liver. The anatomic situation of the adrenals is such that their epinephrin discharge is equivalent to an intravenous injection in the systemic, not the portal, domain, and this arrangement therefore seems in all respects least adapted to act upon the liver glycogen. It is therefore possible that the hyperglycemia and glycosuria from epinephrin injections represent a purely artificial drug effect, not corresponding to anything in either normal or pathological metabolism. The argument that any marked pharmacologic property of a glandular extract must indicate a similar activity on the part of the living gland is best answered by the example given by Kennaway and Mottram,<sup>40</sup> namely, the galactagogue substance which is found in the pituitary glands of fish. If all the above objections be discarded, the question of dosage is still too important to be ignored. The quantities of epinephrin used to produce glycosuria are huge in a physiological sense. It is doubtful if any such quantities are thrown into the circulation from the adrenals under either normal or abnormal conditions, and it is certain that if such quantities are ever poured into the veins they must cause elevated blood pressure and other disturbances. No such thing as a prolonged continuous epinephrin glycosuria has ever been produced, and the idea is absurd on the face of it, because of the renal injuries and systemic intoxication which must result from the large quantities required for this purpose in either normal or potentially diabetic animals. On the other hand, small doses of epinephrin lower the blood pressure instead of raising it,<sup>41, 42, 43, 44</sup> and likewise lower the blood sugar instead of raising it.<sup>45</sup> Repeated small doses of epinephrin increase glycogen storage.<sup>46</sup> These interesting results of small quantities, which perhaps fall within the limits of physiological epinephrin production, have been completely overlooked in the current speculations on the subject.

4. Function of the Chromaffin Tissue and Epinephrin.—Several facts are opposed to a specific antagonism between epinephrin and insulin. First is the different action of different quantities of epinephrin just mentioned. Second, if it should be granted that the acute disturbance following double epinephrectomy is in any way due to deficiency of epinephrin, it could still be pointed out that the pluriglandists as usual have seized upon the one feature which suits their views, namely, the hypoglycemia, and have ignored the other equally striking occurrence, namely, the rapid disappearance of glycogen and loss of power to form glycogen,<sup>47, 48</sup> which is the direct opposite of the results of insulin excess. Third, the effect of epinephrin as an antidote to an overdose of insulin, which seems so striking, represents merely sudden glycogenolysis, entirely comparable to such an artificial procedure as a glucose injection. It is not only susceptible to interpretation as an unphysiologic drug action, as above mentioned, but also is apparently dependent upon the presence of glycogen. Epinephrin probably cannot antidote an overdose of insulin in a diabetic who lacks sufficient glycogen.<sup>49</sup> Absence of insulin results in glycosuria which is not dependent upon glycogen but continues at the expense of maximum sugar formation from protein. This fact alone warrants the inference that epinephrin is not a specific antagonist of insulin, and that deficiency of the one hormone is not equivalent to excess of the other. From a broader viewpoint, attention may be directed to the results of all the recent investigations of the function of the chromaffin tissue and epinephrin. The views of Cannon and Stewart, opposed in several particulars, are fully harmonious on this point. Stewart's criticisms have exposed the speculative and erroneous opinions which have been so widely entertained concerning the role of the adrenals, and his researches and those of his pupils<sup>50</sup> to<sup>66</sup> have tended to discredit epinephrin excess as an explanation of various glycosurias and other experimental phenomena, and thus to shatter the entire pluriglandular conception of this question. Cannon<sup>67, 68, 69, 70</sup> and others<sup>71, 72, 73, 74</sup> have championed the emergency function of the chromaffin tissue, in the sense that a discharge of epinephrin under a strong nervous stimulus increases muscular tone and also mobilizes extra sugar as fuel for strenuous activity. This provision might be highly advantageous; but, with recognition of the fact that diabetes means inability either to



burn or to store carbohydrate, it is at once evident that any tendency to diabetes would be extremely disadvantageous under these circumstances and would defeat the entire purpose of the mechanism. The entire embryology and phylogeny of the chromaffin system indicate some functional association with the sympathetic nervous system, which is something altogether apart from diabetes.

5. Nature of Diabetes.—The basic characteristic of diabetes is the inability to utilize food, particularly carbohydrate. An overproduction of sugar may be a usual accompaniment, but clear thinking must classify a depancreatized animal as diabetic to the same degree as before, even if hypoglycemia has been produced by removal of the adrenals or liver or by any profound shock or exhaustion, because insulin and the normal nutritive function which depends upon it are still absent. That epinephrin does not prevent sugar utilization is proved by the retention of a large proportion of any dosage of sugar even at the height of epinephrin glycosuria,<sup>1, 29</sup> and by respiratory studies,<sup>75 to 79</sup> particularly those of Lusk.<sup>80, 81</sup> Most persons who lightly accept an antagonism between epinephrin and insulin fail to recognize that this is one of four doctrines of the Vienna school which are inseparably bound together. First, diabetes is supposed to be a pure overproduction of sugar, without any impairment of utilization; the glycosuria merely conforms to Pflüger's idea of "a glass running over." Second, the liver is depicted as the "sugar factory," in which the machinery of production is driven by epinephrin, while the function of insulin is merely to act as a "brake" to hold this production within normal limits.<sup>82, 83</sup> Third, the doctrine of sugar formation from fat is an essential feature, because the alleged quantities of sugar, far in excess of anything derivable from carbohydrate or protein, can come from no other source. Fourth, the above views require denial of the generally accepted explanation of acidosis; therefore it is asserted that the acetone bodies are by-products of the formation of sugar from fat in the liver. These four Vienna doctrines, however unproved and pernicious, are at least consistent. Anyone, however, who accepts scientific proofs must recognize that the excess of sugar in diabetes is an expression of the profound nutritive disturbance. The function of insulin is not merely to build up glycogen or prevent sugar formation, but to provide



for the normal utilization of carbohydrate and other foods and for the entire bodily nutrition. This specific nutritive function of insulin, and the reinforcement of sympathetic nervous activities by epinephrin, are processes of a totally different order. Glucose injections, which cause hyperglycemia, are not antagonistic to insulin, but on the contrary are utilized by means of insulin. Likewise epinephrin, piqure, ether and all other agencies which cause hyperglycemia by glycogenolysis cannot be regarded as antagonistic to insulin, unless they tend to create true diabetes by abolishing the utilization of sugar. In the same sense, it is ridiculous to regard liver removal, shock, poisoning by peptone or hydrazine, or other causes of hypoglycemia as adjuvants to insulin or antagonists of epinephrin. The whole question comes down to the usual fallacy of the confusion between the disease, diabetes, and the mere symptom of glycosuria or hyperglycemia. It is like reverting to a stage of medicine in which all forms of fever are regarded as identical, and every means of reducing body temperature is supposed to counteract the cause of the fever. As long as the prostration of sugar utilization and of the entire normal nutrition stands as the essential characteristic of diabetes, no agent which merely causes glycogenolysis or hyperglycemia can properly rank as a physiological antagonist of insulin.

## *II. Abnormal Lability of Diabetic Tissues.*

The fact which leads deepest into the understanding of diabetes is the influence of undernutrition and overnutrition. The insulin consumption of the body is affected by the total calories of the diet and by the body weight.<sup>84</sup> The latter factor is the more surprising, but it is established beyond mistake that obesity conduces to bring on active diabetes and that patients and animals tolerate higher diets and require smaller insulin dosage at low than at high body weights. Totally depancreatized animals not only suffer a rapid progressive breakdown of their tissues, but also show a lack of power to heal wounds or resist infection which is not explainable by simple glycosuria, hyperglycemia or cachexia. This condition is probably analogous to the well-known vulnerability of the tissues of patients with active diabetes, as manifested by numerous infectious complications, such as carbuncle and gangrene, and by the non-infectious complications, such as cataract, retinitis, arteriosclerosis, etc. Control of the

diabetes by diet, even when this involves undernutrition to the point of cachexia, confers practically absolute immunity against all complications.<sup>85</sup> Therefore, in some way, the tissues are healthier with the low nutrition of inanition than with the specific malnutrition of active diabetes. The writer formerly<sup>86</sup> brought evidence that the Langerhans hormone is concerned not only in catabolism but also in anabolism and the upbuilding and maintenance of the entire body mass. Though the storage of insulin in the body must be small, as proved by the quick onset of diabetes after pancreatectomy, there must be some explanation of the remarkable fact that an organism at a very high level of weight seems to use up actually several times as much insulin as the same organism at a very low level of weight.<sup>84</sup> A corollary of the above hypothesis is that deficiency of insulin must create difficulty in building up or repairing tissue and a tendency to breakdown of existing tissue. The signs of impairment of resistance and nutrition in all parts of the diabetic body are thus easily explained. Tests of this hypothesis cannot be made with substances which introduce an element of cachexia or otherwise interfere with sugar formation, but they are to some extent possible with substances which tend to increase sugar formation. Such tests may give some indication whether the living protoplasm of the diabetic organism is more readily broken down into sugar than that of the non-diabetic organism.

The experiments with epinephrin in normal dogs, in partially depancreatized dogs without active diabetes, and in partially depancreatized dogs with active diabetes, showed the following results:

(a) In the normal animals epinephrin produced the familiar hyperglycemia and glycosuria, derived evidently from the glycogen stores as commonly understood, and with no indication of direct sugar formation from protein. The total sugar excretion was small, and the assertion was ventured that no form of administration can be devised which can maintain heavy continuous glycosuria, because of the fatal intoxication which must quickly result from the doses of epinephrin required for this purpose.

(b) The only possible contention of the upholders of glandular antagonisms must be that with deficiency of insulin the

influence of epinephrin upon sugar production is exaggerated, so that heavy continuous glycosuria may be kept up, even at the expense of protein decomposition, by quantities of epinephrin which do not cause elevation of blood pressure or toxic symptoms in the typical diabetic. Dogs D4-77 and E5-00 were severely diabetic, as proved by the low diets required to bring their diabetes under control. Dog F6-07 had only one-eleventh of the pancreas, but sugar freedom was maintained by fasting before and after the operation. Dog D4-77 received slow intravenous injections of epinephrin in dosage which produced obvious effects upon the cardiac action and blood pressure, though no record of these was kept. No glycosuria resulted. All these dogs received epinephrin by the methods which are most effectual for glycosuria, namely, subcutaneous or intraperitoneal injections, but the glycosuria was slight and transitory, with no evidences of progressive tendencies or overproduction of sugar from protein. These experiments seem to be fairly crucial regarding the pluriglandular hypothesis, because if epinephrin is a true antagonist of insulin it should produce some tendency to diabetes under these conditions. If it places the Langerhans tissue under strain, the few remaining islands should show hydropic changes. If it in any way inhibits or paralyzes the action of insulin, marked diabetic symptoms should result with such a scanty insulin supply. If it can reproduce the chief characteristic of diabetic glycosuria, namely the excretion of sugar derived from protein, this power should be plainly manifest when the alleged "brake" action of insulin is thus weakened. The negative results under all these heads appear conclusive.

(c) Absence of glycosuria may be taken as an indication that the organism possesses at least the minimum amount of insulin necessary to prevent the abnormal breakdown of its tissues to form sugar. Correspondingly, diabetics free from glycosuria are ordinarily immune to complications. Therefore a demonstration of an abnormal lability of diabetic tissues can be anticipated only with a deficiency of insulin sufficient to cause active symptoms. In the actively diabetic dog F6-06, epinephrin injections produced a great increase of both sugar and nitrogen excretion, as compared with the various controls; namely, F6-08 and F6-11, fasting normal or diabetic animals without active symptoms; F6-10, a fasting normal dog receiving adrenalin injections; F6-07,

a fasting diabetic dog without active symptoms, receiving adrenalin injections; and F6-09, a fasting diabetic dog with active symptoms without adrenalin. The increase of nitrogen in dog F6-06 is specially important as showing that the extra glycosuria represented not merely a sweeping out of preformed carbohydrate but an actual increase of protein destruction extending over a number of days. Eppinger, Falta and Rudinger reported an increase of glycosuria from adrenalin in a totally depancreatized dog. A choice may be necessary between two explanations, namely the pluriglandular view of an epinephrin element in diabetes, and the hypothesis of an abnormal lability of diabetic tissues. Regarding the first, it must be recognized that the symptoms of existing diabetes may be aggravated by many agencies (carbohydrate excess, thyroid intoxication, systemic infections, etc.) which have not the slightest influence as primary causes of diabetes. Also, it is believed that the previous experiments and discussion sufficed to eliminate epinephrin as a direct antagonist of insulin or a diabetogenic agent.

In any event, the observations show that epinephrin injections caused an excess elimination of sugar and nitrogen in an animal with active diabetes, out of all proportion to the results in normal or potentially diabetic controls. Such experiments are open to different interpretations, and do not prove unequivocally that the tissues with active diabetes are abnormally subject to breakdown. Owing to the incidental difficulties, several other attempts of this kind failed and only this one series was carried through satisfactorily, so that confirmation in a larger number of animals is desirable. The results obtained, however, at least harmonize well with the other evidence of the role of insulin in upbuilding and maintenance of the body and the abnormal lability of tissues arising from the lack of insulin.

### CONCLUSIONS.

1. Removal of most of the adrenal tissue of dogs did not alter the carbohydrate tolerance as judged by intravenous injections of glucose. The tendency to diabetes was also unaffected, apart from the influence to be expected from any fatal cachexia.

2. Fat feeding created no increased tendency to epinephrin glycosuria. A resemblance to diabetic glycosuria in this respect was therefore not demonstrable.



3. Epinephrin injections over a series of days failed to produce active diabetes in dogs which already had severe latent diabetes in consequence of extensive pancreatectomy. The glycosuria was only a trifle greater than in normal animals with the same dosage, and was evidently due as usual to glycogen breakdown, as no indications of overproduction of sugar from protein were found. Tendencies to progressiveness, or signs of functional strain in the form of vacuolation of the remaining Langerhans island cells, were also not produced by epinephrin.

4. The above evidence, together with much already existing in the literature, is interpreted as proving that epinephrin is not a diabetogenic agent or a physiological antagonist of insulin.

5. In an animal with active diabetes, epinephrin injections caused a greatly increased excretion of both sugar and nitrogen, as compared with normal or diabetic controls. The most important feature was the extra destruction of protein continuing over a number of days, indicating that the increased glycosuria was not a mere sweeping out of preformed carbohydrate. No positive interpretation is established, but this result harmonizes with other evidence of an anabolic role of insulin, which is essential for upbuilding and maintaining the entire body mass, and of an abnormal susceptibility to breakdown of the tissues with active diabetes.

#### REFERENCES.

1. Allen, F. M. *Studies concerning glycosuria and diabetes*. Harvard University Press, 1913, Chapters XVI and XIX.
2. Porges, O. *Ztschr. klin. Med.*, 69, 1909-10, 341-9. Ueber Hypoglykämie bei Morbus Addison sowie bei nebennierenlosen Hunden.
3. Porges, O. *Verh. dtsch. Kong. f. inn. Mediz.*, 27, 1910, 591-93. Ueber den Einfluss der Nebennieren auf den Kohlehydratstoffwechsel.
4. Bernstein, S. *Berl. klin. Woch.*, 1911, 1794-6. Ueber den Blutzucker-gehalt bei Addisonscher Krankheit.
5. Forscbach and Severin. *Arch. exp. Path. u. Pharm.*, 75, 1914, 168-193. Verhalten des Kohlehydratstoffwechsels bei Erkrankungen von Drüsen mit innerer Sekretion.
6. Fry, H. J. B. *Quarterly Journal of Medicine*, 8, 1914-15, 276-299. The pituitary gland in diabetes mellitus and disorders of the glands of internal secretion.
7. Rosenow, G., and Jaguttis. *Klin. Woch.*, 1, 1922, 358-360. Der Blutzucker bei Addisonscher Krankheit und seine Beeinflussung durch Adrenalin.



8. Frouin, A. *C. r. Soc. Biol.*, 64, 1908, 216-217. Ablation des capsules surrénales et diabète pancréatique.
9. Mayer, A. *C. r. Soc. Biol.*, 64, 1908 (1), 219-21. Ablation des surrénales et diabète pancréatique.
10. Mackenzie, G. M. *Arch. Int. Med.*, 19, 1917, 593-610. The suprarenal system and carbohydrate metabolism.
11. Hédon, E., and Giraud, G. *C. r. Soc. Biol.*, 83, 1920, 1310-1312. Relation entre le pancréas et les capsules surrénales au point de vue de diabète.
12. Lépine, R. *Le Sucre du Sang*, Paris, 1921.
13. Hoskins, R. G., and Wheelon. *Amer. J. Physiol.*, 34, 1914, 172.
14. Hoskins, R. G. *Amer. J. Physiol.*, 36, 1914-15, 423-429. The effect of partial adrenal deficiency upon sympathetic irritability.
15. Mann, F. C., and Drips, Della. *Arch. Int. Med.*, 16, 1915, 681-692. The relation of the adrenals to the pancreas.
16. Stewart, G. N. *Endocrinology*, 5, 1921, 283-306. Adrenal insufficiency.
17. Houssay, B. A., and Lewis, J. T. *C. r. Soc. Biol.*, 85, 1921, 1210-1212. Importances comparatives des parties médullaire et corticale des surrénales.
18. Lewis, J. T. *Amer. J. Physiol.*, 64, 1923, 503-505. Extirpation of adrenal glands in albino rats.
19. Crowe, S. J., and Wislocki, G. B. *Bull. Johns Hopkins Hosp.*, 25, 1914, 287-304. Experimental observations on the suprarenal glands with especial reference to the functions of their interrenal portions.
20. Houssay, B. A., and Lewis, J. T. *C. r. Soc. Biol.*, 85, 1921, 1212-1213. Diabète pancréatique chez les chiens privés de la partie médullaire des surrénales.
21. Houssay, B. A., and Lewis, J. T. *Amer. J. Physiol.*, 64, 1923, 512-521. The relative importance to life of cortex and medulla of the adrenal glands.
22. Allen, F. M., and Wishart, Mary B. *J. Biol. Chem.*, 42, 1920, 415-458. Intravenous glucose tolerance of dogs.
23. Allen, F. M. *J. Metabolic Research*, 1, 1922, 619-665. Experimental Studies in Diabetes. 10. The influence of the thyroid upon diabetes.
24. Allen, F. M. *Amer. J. Med. Sci.*, 161, 1921, 350-365. Pancreatic cachexia.
25. Blum, F. *Pflügers Arch.*, 90, 1902, 617-629. Weitere Mittheilungen zur Lehre von dem Nebennierendiabetes.
26. Roubitschek, R. *Pflügers Arch.*, 155, 1913, 68-76. Zur Frage der Zuckerbildung aus Fett.
27. Eppinger, Falta, and Rudinger. *Ztschr. klin. Med.*, 66, 1908, 1-52; 67, 1909, 380-398. Ueber die Wechselwirkungen der Drüsen mit innerer Sekretion.
28. Allen, F. M. *Amer. J. Med. Sci.*, 153, 1917, 313-362. The role of fat in diabetes.
29. Underhill, F. P., and Closson, O. E. *Amer. J. Physiol.*, 17, 1906-07, 42-54. Adrenalin glycosuria, and the influence of adrenalin upon nitrogenous metabolism.

30. Underhill, F. P. *J. Biol. Chem.*, 9, 1911, 13-18. The influence of urethane in the production of glycosuria in rabbits after the intravenous injection of adrenalin.
31. Allen, F. M. *J. Metabolic Research*, 1, 1922, Nos. 1 and 2. The pathology of diabetes.
32. For references, cf. (1), p. 690.
33. Tuffier, E. *Rev. d. therap. med. chir.* (Paris), 81, 1914, 399. Le virilisme surrénale.
34. Blanchard, R. *Bull. Acad. Med.* (Paris), 76, 1916, 47. Le virilisme et l'inversion des caractères sexuels.
35. Elliott, T. R. *J. Physiol.*, 49, 1914-15, 38-53. Some results of excision of the adrenal glands.
36. Bittorf. *Münch. med. Woch.*, 1911, 2213. Ist beim Diabetes mellitus eine Ueberfunktion der Nebennieren nachweisbar?
37. Janeway, T. C., and Park, E. A. *J. Exp. Med.*, 16, 1912, 541-57. The question of epinephrin in the circulation and its relation to blood pressure.
38. Allen, F. M., and Sherrill, J. W. *J. Metabolic Research*, 2, 1922, 429-545. The treatment of arterial hypertension.
39. (1), p. 705.
40. Kennaway, E. L., and Mottram, J. C. *Quart. J. Med.*, 12, 1919, 225-258. Observations upon two cases of diabetes insipidus; with an account of the literature relating to an association between the pituitary gland and this disease.
41. Moore, B., and Purinton, C. O. *Pfügers Arch.*, 81, 1900, 483-490. Ueber den Einfluss minimaler Mengen Nebennierenextracts auf den arteriellen Blutdruck.
42. Hoskins, R. G., and McClure, C. W. *Arch. Int. Med.*, 10, 1912, 343-356. The adrenal glands and blood pressure.
43. Cannon, W. B., and Lyman, H. *Amer. J. Physiol.*, 31, 1912-13, 376-398. The depressor effect of adrenalin on arterial pressure.
44. Collip, J. B. *Endocrinology*, 6, 1922, 402-408. Some factors which modify the epinephrine reaction.
45. Weinberg. *Münch. med. Woch.*, 69, 1922, 797. Adrenalinwirkung auf Blutdruck und Blutzucker bei verschiedener Konzentration.
46. Pollak, L. *Arch. exp. Path. u. Pharm.*, 61, 1909, 149-73. Experimentelle Studien über Adrenalin-Diabetes.
47. Porges, Otto. *Ztschr. klin. Med.*, 70, 1910, 243-250. Zur Pathologie des Morbus Addison. II. Ueber Glykogenschwund nach doppelseitiger Nebennierenexstirpation bei Hunden.
48. Mackenzie, G. M. *Arch. Int. Med.*, 19, 1917, 593-610. The suprarenal system and carbohydrate metabolism.
49. Allen, F. M., and Sherrill, J. W. *J. Metabolic Research*, 2, 1922, 803-985. The use of insulin in diabetic treatment.
50. Stewart, G. N., Rogoff, J. M., and Gibson, F. S. *J. Pharm. and Exper. Therap.*, 8, 1916, 205-245. The liberation of epinephrin from the adrenal glands by stimulation of the splanchnic nerves and by massage.

51. Stewart, G. N., and Rogoff, J. M. *J. Pharm. and Exp. Therap.*, 8, 1916, 479-524. The spontaneous liberation of epinephrin from the adrenals.
52. Stewart, G. N., and Rogoff, J. M. *J. Pharm. and Exper. Therap.*, 9, 1916-17, 393-410. The proportion in which adrenalin distributes itself between corpuscles and serum in relation to the technique of testing for epinephrin in blood.
53. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 44, 1917, 149-170. The relation of the rate of the spontaneous liberation of epinephrin to the rate of blood flow through the adrenals.
54. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 44, 1917, 543-580. The alleged relation of the epinephrin secretion of the adrenals to certain experimental hyperglycemias.
55. Stewart, G. N., and Rogoff, J. M. *J. Pharm. and Exper. Therap.*, 10, 1917-18, 1-48. Quantitative experiments on the liberation of epinephrin from the adrenals after section of their nerves, with special reference to the question of the indispensability of epinephrin for the organism.
56. Stewart, G. N., and Rogoff, J. M. *J. Pharm. and Exper. Therap.*, 10, 1917-18, 49-72. The influence of asphyxia upon the rate of liberation of epinephrin from the adrenals.
57. Stewart, G. N. *Amer. J. Physiol.*, 45, 1917-18, 92-95. A note on some obvious consequences of the high rate of blood flow through the adrenals.
58. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 46, 1918, 90-116. The relation of the adrenals to piqure hyperglycemia and to the glycogen content of the liver.
59. Rogoff, J. M. *J. Lab. and Clin. Med.*, 3, 1918, 209-219. On the liberation of epinephrin from the adrenal glands.
60. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 48, 1919, 397-410. Further observations showing that epinephrin from the adrenals is not indispensable.
61. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 51, 1920, 366-377. Further observations on the relation of the adrenals to certain experimental hyperglycemias (ether and asphyxia).
62. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 56, 1921, 213-219. The epinephrin output estimated by collecting the adrenal blood without opening the abdomen.
63. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 56, 1921, 220-229. Post-operative depletion of the epinephrin store of the adrenals.
64. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 62, 1922, 93-112. Morphine hyperglycemia and the adrenals.
65. Stewart, G. N., and Rogoff, J. M. *Amer. J. Physiol.*, 63, 1923, 436-478. The supposed relation of the adrenals to reflex volume changes in the denervated limb.
66. Rogoff, J. M., and Coombs, Helen C. *Amer. J. Physiol.*, 64, 1923, 44-74. Observations on the supposed relation of the adrenal glands to the blood pressure response during cerebral anemia.

67. Cannon, W. B. *Amer. J. Physiol.*, 32, 1913, 44-60. The effect of adrenal secretion on muscular fatigue.
68. Cannon, W. B. *Amer. J. Physiol.*, 33, 1914, 356-372. The emergency function of the adrenal medulla in pain and the major emotions.
69. Cannon, W. B. *Bodily Changes in Pain, Hunger, Fear and Rage*. Appleton & Co., New York, 1915.
70. Cannon, W. B., and Carrasco-Formiguera, R. *Amer. J. Physiol.*, 61, 1922, 215-227. Studies on the conditions of activity in endocrine glands. XI. Further evidence for reflex and asphyxial secretion of adrenin.
71. Carrasco-Formiguera, R. *Amer. J. Physiol.*, 61, 1922, 254-269. The production of adrenal discharge by piqure.
72. Hartman, F. A. *Endocrinology*, 6, 1922, 511-518. The relation of the adrenals to muscular activity.
73. Hartman, F. A., McCordock, H. A., and Loder, M. M. *Amer. J. Physiol.*, 64, 1923, 1-34. Conditions determining adrenal secretion.
74. Macleod, J. J. R., and Pearce, R. G. *Amer. J. Physiol.*, 29, 1911-1912, 419-35. Studies in experimental glycosuria. VIII. The relationship of the adrenal glands to sugar-production in the liver.
75. Hari, P. (with Lévi, L.) *Biochem. Ztschr.*, 38, 1912, 23-45. Ueber den Einfluss des Adrenalins auf den Gaswechsel.
76. Fuchs, D., and Röth, N. *Ztschr. exp. Path. u. Therap.*, 10, 1912, 187-90. Untersuchungen über die Wirkung des Adrenalins auf den respiratorischen Stoffwechsel.
77. Wilenko, G. G. *Biochem. Ztschr.*, 42, 1912, 44-58. Über den Einfluss des Adrenalins auf den respiratorischen Quotienten und die Wirkungsweise des Adrenalins.
78. Bernstein, S. *Ztschr. exp. Path. u. Ther.*, 15, 1914, 86-115. Studien über die Wirkung einzelner Blutdrüsenextrakte, insbesondere auf den respiratorischen Stoffwechsel, nebst Bemerkungen über den respiratorischen Stoffwechsel bei Blutdrüsenkrankungen.
79. Marine, D., and Lenhart, C. H. *Amer. J. Physiol.*, 54, 1920-21, 248-260. The influence of glands with internal secretions on the respiratory exchange.
80. Lusk, G. *Proc. Soc. Exp. Biol. and Med.*, 11, 1913-14, 49-50. The influence of epinephrin on carbohydrate metabolism.
81. Lusk, G., and Riche, J. A. *Arch. Int. Med.*, 13, 1914, 673-681. Animal calorimetry: Paper VIII. The alleged influence of the adrenals on diabetic metabolism.
82. von Noorden, C. *Der Diabetes mellitus*, Berlin, 1912.
83. von Noorden, C. *New Aspects of Diabetes; Pathology and Treatment*, New York, 1912.
84. Allen, F. M. *J. Metabolic Research*, 3, 1923, 61-176. The influence of fat and total calories on diabetes and the insulin requirement.
85. Allen, F. M., and Sherrill, J. W. *J. Metabolic Research*, 1, 1922, 377-455. Clinical observations on treatment and progress in diabetes.
86. Allen, F. M. *Amer. J. Med. Sci.*, 161, 1921, 16-32. Changes in assimilation by alterations of body mass.





## EXPERIMENTAL STUDIES IN DIABETES.

Series II. The Internal Pancreatic Function in Relation to Body Mass and Metabolism.

### 12. Diabetes and Phlorizin Glycosuria.

FREDERICK M. ALLEN, M.D.

*From the Hospital of the Rockefeller Institute for Medical Research,  
New York.*

The use of phlorizin is classical, both for experiments concerning metabolism as such, and in attempts to imitate the conditions of true diabetes. It was tried for the present purpose in connection with three questions: (1) the special lability of diabetic tissues; (2) the influence of phlorizin glycosuria upon diabetes; and (3) the comparative effects of phlorizin glycosuria and diabetes.

#### 1. *Special Lability of Diabetic Tissues.*

The question here is the same as in the preceding paper,<sup>1</sup> namely whether it is possible to demonstrate a greater tendency to breakdown in diabetic tissues than in normal tissues under the same conditions. It is known from the early literature<sup>2</sup> and from general experience that the glycosuria from a given quantity of phlorizin differs somewhat with the ready availability of glucose. The same dose, for example, causes greater sugar excretion in an animal on abundant protein diet than on fasting, and still greater on high carbohydrate diet. If, therefore, the tissues break down into sugar more readily in the diabetic than in the normal organism, there is a chance that this difference might be revealed by phlorizin.

One step consisted in comparisons between normal dogs and partially depancreatized dogs free from glycosuria. These were placed either on fasting or on identical diets, and then given single subcutaneous injections of either 1 gm. or 0.5 gm. of phlorizin suspended in oil. Comparisons were made of the subsequent excretion of both sugar and nitrogen. The reproduction of several dozen detailed records may be avoided by the brief statement that no significant differences were found. Irrespective whether dogs have mild or severe diabetes, if their sugar has been thoroughly controlled by diet or fasting, they react to phlorizin

in the same way as normal dogs subjected to the same conditions of nutrition. In other words, no increased lability of the body protein of diabetic animals is demonstrable by this method when the diabetes is under control by diet.

The results are different when active diabetic symptoms are present. The higher sugar excretion, as compared with normal dogs, is then so obvious that again most of the records may be omitted.\* One example has already been published in another connection.<sup>4</sup> It was there shown that in dog F6-02 the D:N ratios were markedly higher after removal of enough pancreatic tissue to produce diabetes, even though the animal was fed before the operation and fasting after it. A mere sweeping out of pre-existing carbohydrate is excluded by the corresponding increase of nitrogen which ordinarily occurs, indicating an acceleration of protein breakdown. Dog D4-60 (Table 1, below) may serve as a sufficient illustration of the differences of sugar and nitrogen output in the same animal according to whether diabetic symptoms were present or absent. Dog D4-49 (Table 2, below) received phlorizin only when the diabetes had led to a practically moribund state, and, though preformed glucose was actively eliminated as indicated by the fall of blood sugar, the usual increase of both sugar and nitrogen excretion was prevented by the cachexia.

These findings are summarized in this cursory manner, partly because they are fairly self-evident, but chiefly because they are inconclusive as proof of the point under consideration. Excessive excretion of sugar and nitrogen produced by phlorizin in diabetic dogs previously free from glycosuria would be strong evidence of an increased lability of body protein in such animals. The positive results in actively diabetic animals are less striking, because they may be interpreted as the mere superposition of two agencies, namely diabetes and phlorizin poisoning, both of which tend to cause glycosuria and azoturia. It can only be said that the results resemble those with epinephrin,<sup>1</sup> and correspond to theoretical reasoning. When the diabetes is under thorough dietary control so that glycosuria and hyperglycemia are absent,

---

\* The chief disturbances which may cause irregular results in such experiments are nephritis and cachexia. The reduction of phlorizin glycosuria by renal disease is well known. Phlorizin still causes glycosuria in the most extreme states of prostration;<sup>3</sup> nevertheless, the quantitative output is less with cachexia than with normal nutrition.

it is rational to suppose that the insulin supply is adequate to prevent any abnormal tissue breakdown. On the other hand, when deficiency of insulin is indicated by active diabetic symptoms, the excessive sugar and nitrogen loss resulting from phlorizin do not demonstrate an abnormal tissue lability but are at least in harmony with such an hypothesis.

## 2. *Influence of Phlorizin Glycosuria upon Diabetes.*

Three facts must be considered in relation to this point.

(a) Phlorizin has no direct influence upon the islands of Langerhans. It does not cause hydropic changes, or prevent such changes when they are in progress.<sup>4</sup> This is merely one of many proofs that phlorizin poisoning is a totally different condition from true diabetes, so that confusion should not be created by applying the name diabetes to it.

(b) It is currently accepted that moderate doses of phlorizin cause no increase of either nitrogenous or total metabolism in animals receiving adequate rations of protein or carbohydrate. In fasting, however, the sugar which is not supplied by food must be derived from the tissues, and a tremendous increase in the nitrogen excretion and the respiratory exchange results.<sup>5</sup> As one purpose in this series of papers is to study the relation between total metabolism and diabetes, this behavior of phlorizin offers one opportunity.

(c) The sugar excreted in diabetes is completely lost to the organism in every sense. It is not burned, and has no influence on protein catabolism, acidosis or any other known phase of bodily chemistry. One of the striking differences between phlorizin glycosuria and diabetes is shown by the behavior of sugar, which escapes combustion but nevertheless affects the metabolism. Glucose spares protein in phlorizinized animals, even though the dose administered is quantitatively excreted.<sup>6</sup> Likewise, the ketonuria of fasting phlorizinized dogs is nearly or completely abolished by protein feeding, even when the entire theoretical glucose content of the protein is lost in the urine.<sup>7</sup> A special problem is thus created in relation to diabetes. Suppose that a diabetic dog is placed on a diet which produces

glycosuria and which would therefore cause inevitable downward progress in the sense of progressive loss of tolerance; but at the same time the animal is given sufficient phlorizin to cause even greater glycosuria than resulted from the diabetes alone. The mere absence of hyperglycemia is not a decisive factor, as shown by the experiments already quoted.<sup>4</sup> The glucose (whether from starch or protein) which is in excess of the animal's tolerance must still be digested and carried through the blood, and it may enter into metabolism in some way indicated by the reduction of protein catabolism and acidosis. Do these processes constitute a functional demand upon the islands of Langerhans, so that the diabetes will still progress? Or does the specific functional demand consist only in the combustion of the sugar, so that phlorizin, by robbing the organism of more sugar than the excess contained in the diet, may actually furnish a means for taking the excessive diet without impairment of tolerance? A similar problem is presented in regard to the facts stated under (b). The nitrogen output and total metabolism of the fasting animal are greatly increased by phlorizin; but at the same time the sugar formed is excreted unburned and the increased metabolism accelerates the course of undernutrition. Which of the seemingly opposed processes actually prevails? Will the increased metabolism break down tolerance, or will the accelerated undernutrition build it up?

The first trials were made in fasting animals. In order to obtain any decisive proof of benefit from phlorizin, the attempt was made to choose dogs with diabetes of such severity that fasting barely failed to arrest it. In the testing of any large number of partially depancreatized dogs, it is not uncommon to find them at such a stage that the glycosuria falls to very small quantities after a few days of fasting, but then ceases to fall further or actually increases with prolongation of the fast. According to that view of diabetes which pays attention only to glucose, it should be a decided benefit to a diabetic animal to have all the glucose withdrawn from its fasting metabolism by phlorizin, so that it is forced to live entirely on non-carbohydrate materials. Such a benefit, however, is either absent or too slight to be demonstrated by the method chosen. It was never possible to show that a diabetes could be cleared up by fasting plus phlorizin when it was resistant to fasting alone.



On the other hand, experiments designed to test the harmlessness of phlorizin easily gave positive results. There is no doubt that animals, whose diabetes is controllable by fasting, respond equally well to the combination of phlorizin and fasting. Some impression was gained that this combination was more beneficial than plain fasting, in arresting the diabetes more rapidly or building up a higher tolerance. Here also definite proof of the benefit cannot be furnished, because no two animals are strictly identical and the diabetes in a single animal is not the same in tests performed at different times; also the tolerance can be judged only after the glycosuria from the phlorizin has ceased. It can, therefore, only be stated positively that the increased metabolism of fasting phlorizinized animals does not interfere with the control of the diabetes. This is an important practical point for the conduct of experiments, such as previously mentioned,<sup>4</sup> to show that active diabetes still causes hydropic degeneration of islands when the blood sugar is kept at a low level by phlorizin. The diabetes must be sufficiently severe to resist the undernutrition entailed by the phlorizin program (with or without fasting), or else the arrest of the active diabetes will naturally arrest the hydropic changes.

The effects of diet with phlorizin can be more definitely shown by protocols, and sample records will therefore be given, as follows:

Dog B2-62. The operative history of this animal has been previously published.<sup>9</sup> On May 22, 1914, approximately two-thirds of the pancreas was removed, and subsequent glucose tests by stomach and subcutaneously showed a tolerance of 8 gm. per kg., slight glycosuria resulting from 9 gm. per kg. Sept. 9, phlorizin was begun in dosage of 0.5 gm. subcutaneously. The dosage was gradually increased as high as 2 gm., sometimes given every day, and sometimes several days apart, in the attempt to maintain the highest possible glycosuria. The sugar excretion remained between 100 and 200 gm. per day. The last phlorizin injection was given on Nov. 18, and glycosuria ceased on Nov. 22. The dog had remained in excellent condition on an unrestricted diet of bread and soup, and the weight of 10.2 kg. was precisely the same at the beginning and end of the experiment. Nov. 23, a test with 9 gm. glucose per kg. showed the same tolerance as before the phlorizin period. Nov. 24, a specimen of pancreas weighing 0.2 gm. was obtained by operation, and microscopically was found normal in islands and acini. The previous report<sup>4</sup> of absence of influence of phlorizin upon the islands of Langerhans is supplemented by this observation of unchanged tolerance and unchanged histologic appearances.



Dog B2-63 was also mentioned in a former publication.<sup>9</sup> Operation on May 22, 1914, left a remnant estimated at 1/6 to 1/7 of the pancreas. For a time glycosuria could be maintained by bread diet with addition of 300 or 400 gm. of glucose daily, but by Aug. 3 the dog refused glucose, glycosuria ceased, and the attempt to break down the tolerance by diet had definitely failed. The diet was then changed to bread and soup without glucose, and phlorizin was begun in dosage of 1 or 2 gm., sometimes daily and sometimes with a day or two between injections. Glycosuria of 100 to 200 gm. daily was thus maintained. Pancreatic tissue weighing 1.32 gm. was removed by operation on the afternoon of Nov. 6, after an injection of 2 gm. of phlorizin that morning. The last phlorizin injection was given on Nov. 14, and glycosuria ceased on Nov. 18. The dog remained in excellent condition and gained weight slightly, from 23.5 kg. at the beginning of the phlorizin period to 23.9 kg. at its close.

The further record is given in the previous publication.<sup>9</sup> The dog's condition was one of extremely mild but permanent diabetes, which slowly became more marked during several months of subsequent carbohydrate feeding. This result is abundantly explained by the amount of pancreatic tissue removed. An even greater effect might have been anticipated had the operation of Nov. 6 been performed before the phlorizin period; therefore, a slight gain of tolerance during this period may be suspected. The tissue removed on Nov. 6 was normal in acini and islands, again illustrating the absence of effect of phlorizin upon the pancreas.

The conditions of this experiment required the dog to eat enough bread to maintain his nutrition and to supply in addition an excretion of 100 to 200 gm. of glucose daily in the urine. The question was whether the extra carbohydrate ingestion under these circumstances would overload the insular function in an animal so close to the verge of diabetes. The answer was in the negative.

Dog D4-60, a large male Irish terrier mongrel, in medium condition at a weight of 19.3 kg., was partially depancreatized on Dec. 14, 1916. The tissue removed weighed 42.5 gm., and the remnant left about the main duct was estimated at 3.7 gm. (1/12—1/13). The resulting severe diabetes was kept under partial control by diet, while tests showed that 500 gm. of beef-lung sufficed to cause glycosuria, and the feeding of 800 gm. caused glycosuria of about 20 gm. daily for 3 days. Beginning Dec. 21, phlorizin was administered in occasional small doses as shown in Table 1. The dog was not catheterized, but the irregularities in the daily urinary analyses due to irregular voiding do not interfere with the purpose of the experiment.

TABLE 1. Dog D4-60  
Comparison of Two Periods on Identical Diet and Phlorizin Dosage

Date	URINE					BLOOD PLASMA			Body Weight kg.	Phlorizin gm.	DIET
	Vol. c.c.	Sugar gm.	T. N. gm.	D:N ratio	Acetone gm.	ACETONE					
						Sugar mg. per 100 cc.	Qual.	Total mg. per 100 cc.			
1916											
Dec. 21	961	21.1	.....	.....	Neg.	361	.....	.....	18.1	0.5	800 gm. lung.
" 21	1542	70.9	.....	.....	"	57	.....	.....	.....	.....	"
" 23	1287	50.2	.....	.....	.....	.....	.....	.....	.....	0.75	"
" 24	1409	83.1	.....	.....	.....	.....	.....	.....	.....	.....	"
" 25	804	50.7	.....	.....	Faint	.....	.....	.....	.....	0.5	"
" 26	728	34.9	.....	.....	Slight	.....	.....	.....	.....	0.5	1 kg. lung.
" 27	764	66.5	18.8	3.52	0.17	81	Neg.	4.0	17.1	.....	"
" 26	1280	83.2	30.0	3.04	0.40	.....	.....	.....	.....	.....	"
" 29	1395	75.2	28.5	2.64	0.22	.....	.....	.....	.....	.....	"
" 30	872	54.9	15.2	3.59	0.15	82	Neg.	.....	.....	.....	"
" 31	991	83.2	25.5	3.25	0.27	.....	.....	.....	.....	.....	"
Jan. 1	408	31.4	11.6	2.72	0.29	.....	.....	.....	.....	.....	"
" 2	309	5.2	1.7	3.08	.....	185	Faint	14.9	15.4	.....	"
" 3	1110	72.2	28.1	2.56	0.52	.....	.....	.....	.....	.....	"
" 4	550	34.8	13.8	2.58	0.52	73	V. Faint	.....	.....	.....	"
" 5	850	61.1	17.4	3.36	0.34	.....	.....	.....	.....	.....	"
" 6	542	21.1	6.2	3.32	0.21	.....	.....	.....	.....	.....	"
" 7	1029	42.2	17.0	2.48	1.02	.....	.....	.....	.....	.....	"
" 8	544	30.5	10.4	2.92	0.37	49	Faint	.....	.....	.....	"
" 9	1055	56.9	8.8	.....	0.25	57	.....	.....	14.9	0.5	"
" 10	1865	121.2	14.9	.....	.....	.....	.....	.....	.....	.....	"
" 11	1295	54.0	10.8	.....	0.28	58	.....	.....	14.5	.....	"

TABLE 2. DOG D4-60

Date	URINE					BLOOD PLASMA		Body Weight kg.	Phlorizin gm.	DIET
	Vol. c.c.	Sugar gm.	T. N. gm.	D:N ratio	Acetone gm.	Sugar mg. per 100 cc.	ACETONE Qual.			
Feb. 7	684	Neg.	.....	.....	Neg.	.....	.....	15.2	0.5	800 gm. lung.
" 8	792	48.8	.....	.....	"	.....	.....	14.6	.....	" " "
" 9	1255	24.2	.....	.....	"	.....	.....	14.9	0.75	" " "
" 10	1120	58.8	.....	.....	"	.....	.....	14.9	.....	" " "
" 11	340	20.9	.....	.....	V Faint	.....	.....	.....	.....	" " "
" 12	1024	62.9	.....	.....	Neg.	98	Faint	16.8	0.5	1 kg. lung.
" 13	866	47.7	21.4	2.23	Faint	.....	.....	14.5	0.5	" " "
" 14	988	47.6	23.6	2.02	Slight	.....	.....	14.1	.....	" " "
" 15	1175	33.9	18.5	1.84	Neg.	.....	.....	14.0	.....	" " "
" 16	621	31.7	13.0	2.44	"	.....	.....	13.9	.....	" " "
" 17	212	Neg.	5.9	.....	"	.....	.....	.....	.....	500 gm. lung. 100 gm. suet
" 18	390	19.6	7.3	2.70	"	.....	.....	14.2	0.5	" " "
" 19	650	36.3	15.0	2.42	"	.....	.....	14.1	.....	" " "
" 20	400	21.0	10.3	2.04	Faint	.....	.....	.....	0.5	" " "
" 21	902	28.6	16.2	1.76	"	.....	.....	14.1	.....	" " "
" 22	919	27.8	16.6	1.54	Mod.	.....	.....	.....	0.5	500 gm. lung. 200 gm. suet.
" 23	1070	37.5	29.7	1.26	"	.....	.....	14.0	.....	" " "
" 24	689	23.8	10.2	2.57	Heavy	.....	.....	13.9	.....	" " "
" 25	885	27.6	15.7	1.76	Mod.	95	Faint	.....	.....	" " "
" 26	642	29.3	7.0	.....	V Faint	.....	.....	13.8	.....	" " "
" 27	836	37.2	6.7	.....	Faint	.....	.....	14.2	.....	" " "
" 28	752	26.2	9.0	.....	"	.....	.....	.....	.....	+150 gm. starch.
Mar. 1	715	40.8	11.0	.....	"	.....	.....	14.5	0.5	500 gm. lung. 200 gm. suet.
" 2	770	30.9	10.8	2.87	V Faint	.....	.....	.....	.....	" " "
" 3	346	17.7	5.7	3.13	"	.....	.....	.....	.....	" " "
" 4	551	17.6	.....	.....	.....	.....	.....	13.7	.....	" " "
" 5	498	17.1	7.7	2.22	Heavy	87	Faint	13.9	.....	" " "

The experiment was planned for trial of a group of opposed factors. The diet was constantly in excess of the known tolerance, but phlorizin robbed the body of more sugar than would have been excreted on account of the diabetes alone. This sugar was entirely derived from protein, except toward the latter part of the experiment when starch was added to burden the tolerance still more severely. Under these circumstances, would the dietary excess, the formation of sugar from protein, etc., break down the tolerance, or would the loss of sugar, due to phlorizin, protect the tolerance? Also, the diet was chosen from general experience as being something near a maintenance ration for a dog of this size, but the phlorizin caused a loss of weight comparable to what occurs in fasting. Under these circumstances it is improbable that any reduction of metabolism occurred, and it is even possible that the total energy exchange was somewhat increased. In other words, by this device the reduction of weight characteristic of fasting was obtained without the characteristic reduction of metabolism. Would there thus be a benefit to the tolerance like that produced by fasting, or not?

These questions could be definitely answered, because an over-feeding program of this duration (Dec. 18 to Jan. 11) in a severely diabetic dog possessing only one-twelfth or less of the pancreas will certainly cause a marked aggravation of the diabetes, so that ordinarily the glycosuria will no longer be controllable by fasting. The last injection of phlorizin was given on Jan. 10. The glycosuria persisted unusually long, and did not cease until Jan. 20, but the plasma sugar was constantly below 0.1 per cent., showing that the phlorizin was still responsible. Meanwhile, following Dec. 11, the protein ration was reduced successively to 300, 200 and 100 gm. of lung, while 200 gm. or more of suet was given daily in order to prevent further undernutrition as far as possible. Normal urine and blood sugar continued with increasing diet after Jan. 20. Jan. 25, the diet was 500 gm. lung and 200 gm. suet. The plasma sugar was 0.113 per cent. before feeding, and rose only to 0.172 per cent. after feeding, while glycosuria remained absent, demonstrating a higher tolerance than before the phlorizin period. Glycosuria continued absent on 800 gm. lung daily with variable quantities of suet, while the body weight rose from a minimum of 13.5 kg. to 14.5 kg. On Feb. 5, the plasma sugar before feeding was 0.094 per cent., and 6 hours after feeding was

0.139 per cent., though this single determination may not have represented the maximum.

The entire experiment up to this point conforms to the rule that the tolerance of the diabetic organism is higher when the weight is reduced. This gain of tolerance was obtained on an actually excessive diet, merely by employing phlorizin to deprive the body of the surplus sugar. The benefit of fasting and reduction of weight was thus achieved without fasting and probably without the accompanying reduction of metabolism.

Probably a number of persons have had some idea of using phlorizin in the clinical treatment of diabetes. The experimental evidence indicates that its use might be feasible in a certain way, by enabling the patient to take a somewhat larger diet while preventing harm to the tolerance by means of this artificial withdrawal of sugar from metabolism. A practical application was never attempted, because of the greater danger of acidosis in human patients than in dogs, and because it appears unwise to create a pathological state, such as phlorizin poisoning, for no better purpose than a possible gratification of appetite.

Following Feb. 7, the experiment was continued for another purpose, namely, the comparison between the action of phlorizin in the later period, when diabetes was only latent, with its action in the earlier period when there had been active diabetes. Accordingly, the exact program of diet and phlorizin was duplicated, as shown in Table 2. The animal in the latter period behaved like a normal dog, showing lower D:N ratios and much lower quantities of excreted sugar and nitrogen than in the earlier period. This result is one of those discussed under section (1) above.

### *3. Comparative Effects of Diabetes and Phlorizin Glycosuria.*

Acidosis was slight both in dog D4-60 and in dog D4-49, described below, and the differences were unimportant. A later publication. (Series V, No. 5) will discuss the peculiarities of acidosis under such circumstances, and also add illustrations of the present topic.

Visible lipemia was absent or trivial in both of these animals. As previously mentioned,<sup>10</sup> however, the intense lipemia of some diabetic dogs and patients is never duplicated in phlorizin poisoning; and though lipemia is not directly related to the insular function, the tendency to it constitutes another difference between diabetes and phlorizin glycosuria.



Totally depancreatized dogs are well known to lack the power of healing wounds or resisting infection to a remarkable degree which seems to be specific to the loss of pancreatic function, for it is not equalled in any other known condition. Partially depancreatized animals recover well from aseptic operations (except cats, which are strikingly deficient in wound healing when nine-tenths or more of the pancreas is removed). Dogs and other species, however, in the later cachectic stages of the diabetes following partial pancreatectomy often show a tendency to ulcers and other infections which apparently are analogous to clinical diabetic gangrene. None of these phenomena are explainable by loss of sugar. "Totally" phlorizinized dogs, though showing higher D:N ratios and higher absolute excretion of sugar than totally depancreatized dogs, withstand operations without anything resembling the prostration of healing and resisting power. Hyperglycemia, or the old idea of "sugar-soaked tissues," is likewise not an explanation, for normal animals subjected to prolonged excess of sugar show no such effects,<sup>11</sup> and the condition of depancreatized animals is not helped by phlorizin. Diabetic animals are peculiarly susceptible to phlorizin, in the sense that their blood sugar is reduced by surprisingly small doses. Illustrations are furnished in a previous paper<sup>4</sup> by the effects of doses as low as 0.2 or 0.3 gm., two or three days apart in the diabetic dog E5-99. Contrary to what might be expected, the plasma sugar was reduced lower in dog D4-60 (Table 1, above) by the same phlorizin dosage in the early period with active diabetes than in the later period with lower body weight and quiescent diabetes. It is similarly possible to control the hyperglycemia of totally depancreatized dogs by harmlessly small doses of phlorizin, but there is not the slightest improvement in the healing or resisting power. This fact should arouse reflection in those who regard diabetes as nothing but a lack of glucose utilization.

Under such a doctrine, it also seems inexplicable that a totally depancreatized dog, if kept free from peritonitis, should still die within one or two weeks, when acidosis is absent or trivial and the animal should theoretically be able to live on non-carbohydrate materials until emaciated to a much greater degree than is found at autopsy. The asthenia of the diabetic dog also seems much more pronounced than that of a fasting phlorizinized dog

under similar conditions. Investigators are mostly familiar with "totally" phlorizinized fasting animals, with the heavy drain upon their nutrition and with acidosis frequently severe. But if the doses of phlorizin are so chosen as merely to duplicate the glycosuria and acidosis of a depancreatized dog, the difference in strength is made more striking.

Depancreatized birds are known commonly to excrete little or no sugar, but will probably be found to die more quickly than phlorizinized birds which have greater glycosuria. Also occasional dogs from unknown causes excrete extraordinarily little sugar after total pancreatectomy, but do not live longer on this account. Further studies of the respiratory quotients in such dogs or in birds may be useful. The symptoms and death following pancreatectomy suggest the possibility that combustion of foods to the normal end products is not necessarily synonymous with normal or beneficial combustion. It is conceivable that the former may occur without insulin, while insulin may be indispensable for actually normal physiological combustion of protein, carbohydrate and fat. The direct participation of insulin in total metabolism is one possible hypothetical explanation why animals are so much worse off when deprived of insulin than when merely deprived of carbohydrate.

A further experimental opportunity seems to be offered by partially depancreatized dogs, which are able to digest and absorb food satisfactorily. A severely diabetic animal typically emaciates in spite of utmost eating and dies with profound weakness and cachexia. Suppose that a non-diabetic animal is placed on the same diet and given such phlorizin dosage as will duplicate the glycosuria, acidosis and any other known chemical abnormalities. Will there be a similar progressive impairment of health and ultimate death?

Several attempts were made to answer this question by parallel observations of diabetic dogs and normal controls treated with phlorizin, but many mishaps can arise to spoil experiments of such a character and duration. The most complete result was obtained in an experiment with dog D4-49, for which dog D4-60 served as a control. Here the most extreme possible conditions were chosen, in two respects. First, both dogs were partially depancreatized, so as to equalize digestive conditions as far as

TABLE 3. DOG D4-49

Date	URINE					BLOOD PLASMA			Body Weight kg.	DIET
	Vol. c.c.	Sugar gm.	T. N. gm.	D:N ratio	Acetone gm.	ACETONE				
						Sugar mg. per 100 cc.	Qual.	Total mg. per 100 cc.		
Dec. 21	860	Heavy	.....	.....	.....	.....	.....	.....	.....	Bread and lung.
" 22	1051	66.2	.....	.....	.....	.....	.....	.....	.....	800 gm. lung.
" 23	1492	91.0	.....	.....	Mod.	.....	.....	.....	.....	" "
" 24	1318	69.9	.....	.....	.....	.....	.....	.....	.....	" "
" 25	755	33.2	.....	.....	Heavy	.....	.....	.....	.....	" "
" 26	1202	67.3	17.6	3.85	"	.....	.....	.....	.....	1 kg. lung.
" 27	1395	84.5	19.3	4.35	0.98	.....	.....	.....	18.0	" "
" 28	1210	37.9	16.1	2.35	0.85	.....	.....	.....	.....	" "
" 29	880	46.4	20.2	2.30	0.84	.....	.....	.....	.....	" "
" 30	961	34.6	14.8	2.35	0.96	.....	.....	.....	.....	" "
" 31	466	21.9	7.5	2.87	0.68	.....	.....	.....	.....	500 gm. lung, 100 gm. suet.
Jan. 1	1045	40.8	16.1	2.54	1.48	384	Slight	27.0	.....	" "
" 2	1470	38.2	13.2	2.89	1.59	.....	.....	.....	.....	" "
" 3	688	37.2	11.2	3.32	1.38	435	Slight	41.5	15.9	" "
" 4	318	22.9	8.8	2.60	0.93	.....	.....	.....	.....	" "
" 5	1315	38.1	18.5	2.06	1.37	.....	.....	.....	15.1	200 "
" 6	982	20.6	12.4	1.66	0.77	.....	.....	.....	.....	" "
" 7	1120	31.4	8.3	3.79	0.93	435	Heavy	52.9	.....	" "
" 8	784	21.0	5.5	3.82	0.67	.....	.....	.....	.....	" "
" 9*	1140	52.4	6.5	.....	0.73	455	.....	21.7	.....	500 gm. lung, 100 gm. suet, and 50 gm. corn starch.
" 10	490	23.3	8.3	.....	0.78	.....	.....	.....	13.6	500 gm. lung, 200 gm. suet.
" 11	225	12.8	3.8	.....	0.29	75	.....	22.0	13.0	" "

\*0.3 gm. phlorizin injected subcutaneously.

possible; and as the diabetes in dog D4-49 had been brought on partly by inflammation, the pancreas remnant was larger than in dog D4-60 and it was hoped the digestion would be correspondingly better. Second, the control dog, D4-60, was actually diabetic, and, as explained under section (2) above, the diabetes had merely been controlled and converted into a different form of glycosuria by means of phlorizin. Could this difference be further demonstrated by the different influence upon the animal, even when the quantities of sugar and nitrogen lost were similar?

The operative record of dog D4-49 was previously published.<sup>12</sup> He was somewhat stronger and heavier than dog D4-60, and weighed 24 kg. on Nov. 24, 1916. On this date 28.5 gm. of pancreatic tissue was removed, leaving the body of the gland, estimated at 15 gm. Glycosuria remained absent until the pancreas remnant was traumatized by crushing in a second operation on Dec. 7. The dog was then unwell and free from glycosuria on account of nearly complete fasting until Dec. 12. With return of appetite and spirits, the eating of considerable bread on that day brought on heavy glycosuria. This continued on a diet of bread and meat, until on Dec. 22 the dog was placed on carbohydrate-free diet for exact comparison with dog D4-60. Owing to the anorexia, this dog had lost more weight than dog D4-60 up to this point, but nevertheless at the beginning of the parallel test dog D4-49 showed distinctly the better strength and general condition of the two.

The pancreatic islands of dog D4-49 were evidently profoundly injured by the inflammation, for they quickly succumbed to hydropic degeneration and at autopsy only rare disappearing remains were found. Correspondingly, the diabetes was not far from "total," as indicated by the D:N ratios (Table 3). The operative wound healed smoothly, but the decline of weight and strength was almost as rapid as after total pancreatectomy. The prostration rapidly became extreme, and unusually early death occurred in profound asthenia on Jan. 11, while dog D4-60 was still in fair strength and spirits. Two days before death (Jan. 9), dog D4-49 was given an injection of 0.3 gm. of phlorizin, which reduced the blood sugar but did not alter the moribund state.

Comparison of Tables 1 and 3 shows that the urinary sugar and nitrogen and D:N ratios of dog D4-49 were fairly comparable with those of the first period of dog D4-60, and the differences in the clinical course are not explainable by any excess of excretion on the part of the former dog. Also, the degree of acidosis was unimportant in both animals. The plasma bicarbonate, which had been normal, fell to 36.6 volumes per cent. in dog D4-49 before death, but there were no symptoms resembling coma, the total acetone of the plasma was too low to be compatible with such a

diagnosis, and the reduction of blood alkali was therefore evidently nothing but a perfectly familiar antemortem occurrence.

Totally depancreatized dogs seem to die before reaching a degree of emaciation equal to that of normal animals which starve to death. But in dog D4-49 and other partially depancreatized dogs, the loss of weight may be sufficient to account for death. Dog D4-60 weighed only 13.3 kg. at the close of the second phlorizin period and was still far from a dangerous condition. Also, the question is still open why a diabetic dog should be weaker than a phlorizinized dog when the weights are similar. Nevertheless, it must be recognized that dog D4-49 lost more weight both absolutely and relatively than the control, and the fatal weakness is at least very largely thus explained.

The diets of the two animals were identical. The feces of both were saved, but circumstances prevented the analyses for nitrogen and fat which had been planned. From the gross appearances it could be inferred that the digestive power of both was closely similar at the outset, but toward the close there may have been progressive failure of digestion in dog D4-49, and this may account for the falling of his urinary nitrogen. This point, however, is not essential. Only respiration tests could prove whether the more rapid wasting of the diabetic dog is due in any degree to a higher metabolism. It would not be surprising if the general failure of bodily powers in a diabetic animal should include a failure of digestion and absorption, and this difference from a phlorizinized animal will be significant if it is constant. The general experience with other observations of this kind, which were not sufficiently complete to report in detail, gives the impression that the difference is constant. Dogs receiving adequate rations of carbohydrate or protein can apparently endure phlorizin glycosuria almost indefinitely without serious harm, while diabetic dogs with similar nitrogen and sugar excretion weaken and die. These observations agree with other evidence of a fundamental difference between the two conditions.

### SUMMARY AND CONCLUSIONS.

1. Partially depancreatized dogs whose diabetes is controlled by diet react to phlorizin like normal animals. Dogs with active diabetes respond to phlorizin with exaggerated sugar and nitrogen excretion. This behavior is in harmony with the idea of an



abnormal tendency to tissue breakdown when insulin is lacking, though it does not prove this hypothesis.

2. When, under suitable conditions, diabetic animals are given a diet in excess of the tolerance, but at the same time are robbed of the excess sugar by means of phlorizin, no impairment of the tolerance results. The loss of weight caused by phlorizin actually raises the tolerance, like ordinary undernutrition. Three deductions are warranted by these experiments:

(a) Phlorizin does not injure the islands of Langerhans.

(b) The digestion and absorption of either carbohydrate or protein, the formation of glucose from protein, the transportation of sugar in the blood and its excretion by the kidneys create no demonstrable demand upon the insular function. This demand seems to arise only from the combustion or storage of sugar or other food.

(c) The rise of tolerance which characteristically accompanies a fall of body weight seems to be obtainable without a reduction of metabolism, since the metabolism during phlorization probably does not fall and perhaps rises. The benefit of undernutrition would therefore seem to consist chiefly in the reduction of body mass. This agrees with other evidence that the maintenance of the entire body mass somehow constitutes a load upon the insular function.

3. Totally depancreatized dogs show loss of healing and resisting power, asthenia, and death before a state of utmost emaciation is reached. Fasting dogs receiving sufficient phlorizin to produce an equal excretion of sugar and nitrogen remain in far better condition. Likewise, partially depancreatized dogs weaken and die with active diabetes, while phlorizinized controls receiving the same diet and excreting similar quantities of sugar and nitrogen survive. Hyperglycemia does not explain the differences, for phlorizin fails to save the diabetic dogs, and in this connection it is noted that surprisingly small quantities of phlorizin suffice to cause hypoglycemia in diabetic animals. Three further conclusions are suggested:

(a) Phlorizin poisoning is a radically different condition from diabetes. The word diabetes is long and thoroughly established as the name of the condition which results from deficiency of

insulin. It therefore appears confusing and scientifically unjustifiable to apply this name to a wholly different condition.

(b) Diabetic symptoms and death are not explainable wholly by the loss of sugar. Animals are much worse off when deprived of insulin than when merely deprived of carbohydrate.

(c) All the evidence seems to characterize diabetes not as a mere deficiency of sugar metabolism but as a specific defect of the total bodily nutrition.

#### REFERENCES.

1. This Journal, p. 589.
2. Allen, F. M. *Studies concerning glycosuria and diabetes*, Harvard University Press, 1913; pp. 623-624.
3. Ibid. p. 621-622.
4. *J. Metabolic Research*, 1, 1922, 75-88. The role of hyperglycemia in the production of hydropic degeneration of islands.
5. Lusk, G. *Ergebn. d. Physiol.*, 12, 1912, 372. *Elements of the Science of Nutrition*, 1917, 474.
6. Ringer, A. I. *J. Biol. Chem.*, 12, 1912, 431-445. Protein metabolism in experimental diabetes.
7. Benedict, S. R., and Osterberg, E. *Proc. Soc. Exper. Biol. and Med.*, 12, 1914, 45. The influence of feeding upon acidosis in the phlorizinized dog.
8. *J. Metabolic Research*, 1, 1922, p. 61.
9. *J. Exper. Med.*, 31, 1920, 433.
10. *J. Metabolic Research*, 2, 1922, 219-298. The production of diabetic lipemia in animals, and observations on some possible etiologic factors.
11. (2), Chapter III.
12. *J. Metabolic Research*, 1, 1922, 179.









134

*Announcement of*  
**The Banting Research Foundation**  
Toronto, Canada



THE discovery and development of Insulin by Dr. F. G. Banting, Mr. C. H. Best and other co-operating investigators has brought relief to a multitude of sufferers from diabetes throughout the world. At a low price this boon has been placed within reach of all. But it is well known that only a beginning has been made in alleviation even of this one malady. Notwithstanding the magnificent advances that have been effected in arresting or averting many of the most grievous attacks of disease on human life, mankind is beset by enemies. Their strategy must be discovered and circumvented. This can be done only by patient research conducted in the main by skilled investigators who devote their lives to scientific inquiry. For these investigators the public at large must provide the means of support, for they it is who benefit immensely thereby. Such work has been going on quietly all over the world. Laboratories in the Universities have groups of investigators working in co-operation under the direction of competent scientists. But only now and then does a result such as Dr. Banting achieved strike the imagination of the world. It is therefore but appropriate that advantage should be taken of it to appeal to the grateful public for support in making possible the continuance and prosecution of this work and of other investigations in medical science. To effect this and to signalize the discovery and the development of Insulin, the Banting Research Foundation has been created.

*The purposes of this Foundation have been defined to be:*

- (a) To provide, in the first instance, further funds for the support of the Banting and Best Chair of Medical Research at the University of Toronto.
- (b) To establish a fund for the adequate financial support of such scientific workers as may have proposed definite problems of medical research, and for whom funds are not otherwise available. Such assistance may be given to persons working in the University of Toronto or elsewhere.

All financial arrangements in connection with the collection and reception of the principal and subsequent expenditure of the income of

the fund have been vested in a Board of Trustees, the members of which are appointed for a term of three years, subject to reappointment at the end of their respective terms of office. Trustees have now been appointed as follows:

SIR ROBERT A. FALCONER, K.C.M.G., D.Litt., LL.D.,  
D.D., Edin.; D.C.L., Oxon., *Chairman*.  
President of the University of Toronto.

LIEUTENANT COLONEL R. W. LEONARD,  
*Honorary Treasurer*.  
Member of the Board of Governors of the University of Toronto.

REV. CANON H. J. CODY, D.D., LL.D.,  
Chairman, Board of Governors, University of Toronto.

C. S. MACDONALD, Esq., M.A.,  
General Manager, Confederation Life Association.

W. E. GALLIE, M.D., F.A.C.S., F.R.C.S., Eng.,  
Surgeon-in-Chief, Hospital for Sick Children, Toronto.

PROFESSOR J. G. FITZGERALD, M.D., F.R.S.C.,  
Professor of Hygiene and Preventive Medicine,  
Director, Connaught Laboratories, University of Toronto.

PROFESSOR V. E. HENDERSON, M.A., M.B.,  
Professor of Pharmacology, University of Toronto.

MR. JOHN W. ROGERS.

The Trustees propose to make an appeal to the public for funds in the immediate future. Subscriptions to the Fund will be welcome at any time and should be made payable to the Banting Research Foundation, Toronto, Canada.

## THE TREATMENT OF DIABETES WITH INSULIN.

A report of the methods followed and the results obtained in the first one hundred cases.

BY

W. D. SANSUM, M.D., N. R. BLATHERWICK, Ph.D., FLORENCE H. SMITH, B.S., M. LOUISA LONG, M.S., L. C. MAXWELL, B.S. ELSIE HILL, B.S., RAY McCARTY, B.S., J. H. CRYST, M.D.

*From the Potter Metabolic Clinic of the Santa Barbara Cottage Hospital, Santa Barbara, California.*

This work was supported in part by a special grant from the Carnegie Corporation of New York.

The work of Banting and Best<sup>1</sup> and their associates in the isolation and clinical use of the sugar-metabolizing hormone, insulin, and its specificity in the treatment of diabetes is now well-known and accepted. There remains the general problem of further perfecting the methods of its use.

Since the advent of insulin, 250 diabetic patients have been admitted to this clinic. Of these, 150 have been severe enough to warrant the use of insulin. It is the purpose of this paper to outline the methods used and record, chronologically, the results obtained in the first one hundred cases.

### *The Sources of the Insulin Used.*

Since April, 1922, we have been preparing an increasing amount of insulin. Our present yield and consumption is equivalent to 50,000 iletin units a week, and, in addition, we are using 10,000 units of the Eli Lilly iletin per week. For home treatment, patients living within a reasonable distance from the clinic, are supplied with iletin through us. Patients living outside of California are supplied directly from Eli Lilly and Company through their local physicians.

In the preparation of our first product, heat was used to circumvent the action of the pancreatic enzymes. In June, 1922, and in advance of publication, the Toronto group generously sent us Collip's method. This is essentially the method we are using at the present time, although we have tried out a number of other methods. Because of our distance from a large

packing plant, the first steps of the process are completed at the Hauser Packing Company's plant in Los Angeles. The pancreatic glands of beeves sheep, and calves are removed as quickly as possible, after the animals are killed, and placed on iced trays. As soon as approximately fifteen pounds of the glands accumulate, they are ground with an electric power-grinder and mixed with equal parts of 95 per cent. alcohol. The mixture is then stirred for one hour in a Read bread-mixer. The brei is next enclosed in inch-thick, cloth-covered molds and pressed, with screens between, by means of a hundred-ton hydraulic press. The screens are then removed and the cakes again pressed. The press juice is further diluted with two parts of 95 per cent. alcohol and shipped to Santa Barbara in ten-gallon milk cans. In the beginning, the material was filtered in the packing plant, but, when filtered immediately, a large amount of insulin adhered to the precipitate. This adherent insulin redissolves in the 80 per cent. alcohol if the mixture is allowed to stand for twenty-four hours or more. The filtration process is then comparatively rapid. For three months, we had the glands frozen at the packing plant and shipped to this laboratory in thermos containers. The yield, by this method, was relatively small and the final extract contained many split protein products. These products of digestion probably arose during the time which elapsed before the glands became frozen entirely solid.

Great care must be exercised to maintain the requisite degree of acidity throughout the process of extraction, concentration and precipitation. Alcohol which has been concentrated from 80 per cent. to 95 per cent. by means of sodium hydroxide is usually quite alkaline. The reaction of such alcohol is adjusted to a pH of about 6.6 to 6.8 by adding a sufficient amount of hydrochloric acid. The 80 per cent. alcohol, used for dissolving the insulin-containing material after concentration, is maintained at a pH of 6.6 to 6.8. Absolute alcohol prepared by dehydration with quicklime is often quite alkaline and it is of the utmost importance to adjust properly the reaction of this in order to obtain the optimum flocculation when the material is thrown out of alcoholic solution. We find that absolute alcohol having a pH of approximately 5.3 is very satisfactory. The method of preparation used is briefly described as follows: From 12 to 15 quarts of extract are placed in a 22 liter balloon flask (Pyrex) and concentrated in vacuo to a volume of about 700 to 800 cc. This distillation may be accomplished in about two hours providing tubing of at least one inch diameter is used for the connections between the flasks and the condensers, and also about half way down the condensers. Smaller tubing retards the rate of distillation by the resistance which it offers to the flow of the vapors. The flasks are immersed in a steam-heated water-bath the temperature of which is automatically maintained at 80° centigrade. The distillate is collected in ordinary five gallon water bottles. The concentrated material is strained through cloth to remove the gross fat which has separated, and is cooled in running water. It is then washed twice with ether to extract the remaining fat. The material collected from our usual run of 39 to 78 quarts is then subjected to a second distillation in vacuo. This distillation takes place from a 12 liter balloon flask. By using the large tubing described and by having the vacuum pump operating only upon

this flask, the temperature within the flask may be maintained at 31° centigrade or below. After the material has become thick, 150 cc. of 80 per cent. alcohol for each twelve quarts of original extract are added and the flask is thoroughly shaken. Centrifugation is done in ordinary round nursing bottles of six ounce capacity (Rosenow serum bottles). Precipitation is effected by adding the extract in 80 per cent. alcohol to 4 volumes of absolute alcohol of pH 5.3. Immediate flocculation should occur. After standing two days, the supernatant alcohol is poured off and the precipitate is redissolved in 80 per cent. alcohol. The solution is filtered to remove the insoluble matter and is again precipitated by adding to 4 volumes of absolute alcohol. After this has stood for two days, the alcohol is decanted and the remaining alcohol is removed by inverting the container and finally by attaching to the vacuum pump. The precipitate is finally taken up in sterile, distilled water containing 0.25 per cent. tricresol. Dilute sodium hydroxide is added to a pH of about 6.8 at which point there is nearly complete solution. The aqueous solution is filtered through paper; passed through a Berkfield of medium fineness; bottled in one ounce vaccine bottles; sterility tests are made, and it is finally standardized by rabbit tests. The final product is perfectly clear and slightly colored. It is non-irritating and entirely satisfactory for clinical use. This product admittedly contains foreign materials which can be removed by suitable procedures, but owing to losses in potency which result, we have adhered to the method as described.

The cost of insulin to patients has been no bar to what we consider its adequate use, since our research work has been well supported financially, and we have been fortunate in securing an insulin subsidy fund. Each patient pays what he can, and the remainder of the actual cost to us is charged to the insulin subsidy fund.

#### *The Treatment of Diabetes with Insulin.*

In the treatment of Diabetes, there are three objects to be attained.

1. The patient should be kept continuously "sugar-free," and the blood sugar should be normal.
2. The patient should be kept continuously free from acidosis.
3. The patient should be nourished as evidenced by a satisfactory weight.

These conditions may be fulfilled, in many instances, by careful dietary procedures, although when a patient's tolerance is very low continuous bed-rest is necessary to avoid a serious loss of weight. If the disease becomes progressively worse, as it usually does in severe and untreated cases, a stage is finally reached when



the patient can no longer be kept "sugar-free" and free from acidosis even if the most careful attention is paid to the diet, and he is kept continuously in bed.

By the use of insulin, the death rate from diabetes may be reduced to zero, if the patients are seen before deep coma has developed, and the patients who would otherwise remain chronic invalids may be restored to health by ample diets in proportion as this specific extract becomes available. At the present cost, it is not available to all. It is not a cure for diabetes. Patients will need to exercise greater care than ever with their diets, but since these diets will be ample for their needs, they will be fully repaid for the additional effort.

We believe that, in the treatment of diabetes with insulin, there are four conditions that should be satisfied.

1. The sugar-burning or utilizing power of the insulin in grams per cubic centimetre should be known.
  2. The patient's natural tolerance should be determined in grams of sugar-formers.
  3. The exact value of the proposed diet should be known.
  4. The dosage of insulin may then be adjusted to make up the difference between the sugar-formers of the proposed diet, and those of the patient's natural tolerance.
1. The sugar-metabolizing power of the insulin should be accurately known.

Eli Lilly's iletin is evaluated in rabbit units. In the beginning, the rabbit unit was defined as the amount of insulin required to lower the normal blood sugar of a one-kilogram rabbit to 0.045%, at which point convulsions usually occur. These units then had a very constant value because of the large number of rabbits used in the evaluation of each lot number. During the months of February, March, April and part of May, such units when tested on patients whose tolerances were known to have remained constant over long periods of time, had a patient value of approximately 1.25 grams of sugar-metabolizing power per unit in their H-20 product. No demonstrable change in the value of the unit could be detected between mild and extremely severe cases, or in the product when used to assist in carrying low or high diets in the same patient. During the month of May, many of our most carefully measured and trained patients began to pass sugar in the urine, and found it necessary either to reduce their diets or

increase their iletin in order to remain constantly aglycosuric. Some patients even returned to the clinic to ascertain if there were errors in their diets. No dietetic errors were found, and when such patients were given some of our own proven lot numbers, their tolerances were found to be unchanged. The patient value of the iletin unit appeared to be about two-thirds of what it had been in the past, or in the neighborhood of 0.85 grams per unit, or 17 grams per cc. of the H-20 product. We then learned that the unit had been redefined<sup>3</sup> as being one-third of the amount required to lower the blood sugar below 0.045% and cause convulsions in a two-kilogram rabbit which had been previously starved for twenty-four hours. This redefinition was based on the belief, supported by experimental evidence, that it requires four times as much insulin to cause a convulsion in a two kilogram rabbit as in a one kilogram rabbit. In using one-third instead of one-fourth the convulsion does in the two kilogram rabbit, these workers believed that they were increasing the value of the unit. In the experimental evaluation of our own product, we have found that the convulsion dose varies directly with the weight of a rabbit that has been kept on a standard diet. By using a diet of alfalfa, we have found the blood sugar very constant, whereas in the past with a mixed diet of alfalfa and crushed barley, the blood sugars were inconstant and often very high.

In our experience, the present commercial iletin is a very constant product worth approximately 0.85 grams per unit or 17 grams per cc. of the H-20 product. Eli Lilly and Company also manufacture H-5, H-10 and H-40 strengths.\* We are now using some of the H-40 product and find it has a patient value of approximately 34 grams per cc.

2. The patient's tolerance may be determined by diet alone or by diet plus insulin. When diet alone is used, the patient is desugarized by partial starvation. When the patient is "sugar-free" diet additions are gradually made. A diet is eventually found upon which the patient can remain continuously free from sugar in the urine and with a normal blood sugar. In evaluating diets and estimating tolerances, it is much simpler to reduce the various food factors to the common denominator, sugar-formers. The sugar-formers of the diet, designated by "G," (Woodyatt)<sup>4</sup> are 100% of the carbohydrate, 58% of the protein and 10% of the fat.

\* At the time this paper was submitted for publication, Eli Lilly and Company were still marketing the H-insulin and had not yet changed to the U strength.

Suppose that the maximum diet upon which a patient can remain continuously "sugar-free" and with a normal blood sugar contains 35 grams of carbohydrate, 38 grams of protein, and 83 grams of fat. The sugar-formers of such a diet are 100% of 35, 58% of 38, and 10% of 83, or 35 plus 22.04 plus 8.3, or 65.34 grams. We would say that such a patient has a natural tolerance of 65 grams of sugar formers.

In the terminal stages of diabetes, patients do not endure starvation well because of the existing acidosis and their already marked emaciation. Many of them cannot be desugarized by any dietary procedure since their natural tolerances are too low to permit of even bed-rest, maintenance diets. In such cases, tolerances may be determined by diet plus insulin. If the acidosis is not too severe, the patient may be given a bed-rest, maintenance diet similar to the above, which contains a little more than 1000 calories, with 65 grams of sugar-formers. Small doses of insulin, 20 grams of sugar-metabolizing power, are given at first. The insulin is gradually increased until the patient is continuously "sugar-free." Suppose that it requires 60 grams of assistance in the form of insulin to carry the above diet. The patient's natural tolerance would then be 65 minus 60 or 5 grams of sugar-formers. If the patient has a severe acidosis no attempt is made to measure him until the acidosis is controlled. This is done by giving the patient a diet rich in carbohydrate, low in protein and as free as possible from fat; for example, oatmeal, skimmed milk and orange juice. At the same time, comparatively large doses of insulin are given and, as the carbohydrate is metabolized, it in turn oxidizes the fat and thus dissipates the acidosis.

### 3. The exact value of the diet should be known.

We consider 80 to 100 grams of protein as ample for the needs of an adult. In adjusting the fat, we have followed the formula of Woodyatt's optimal diets, never letting the fat actually oxidized exceed two times the carbohydrate plus one-half of the protein. Following this plan a diet "G" of 65 will carry a 1000 calorie diet. Where it is desirable to cause an emaciated patient to gain weight as rapidly as possible and while the patient is steadily increasing in weight, we usually add 300 calories of fat in excess of the diet balanced by the Woodyatt formula. This does not change the glucose, fatty acid ratio because the fat which increases the weight of the patient in no way affects this ratio.

#### 4. The adjustment of the dose.

Suppose that the natural tolerance of the patient, as determined either by diet or by diet plus insulin, is found to be 65. Suppose that the proposed diet contains 133 grams of sugar-formers. The patient will need 133 minus 65, or 68 grams of assistance. 68 divided by 17, which is the value of one cc. of the H-20 iletin, will then equal the number of cc. required, or 4 cc. In our experience, we have found in the majority of patients, with an equal distribution of the food between the three meals of the day, that  $\frac{5}{8}$  of the total dose should be given before breakfast and  $\frac{3}{8}$  before supper. In this instance, we would give 2.5 cc. before breakfast and 1.5 cc. before supper. Milder patients may be given one dose per day. The food may be so distributed that the breakfast may fall within the patient's natural tolerance, when the noon dose may be made sufficiently large to carry the excess of dinner and supper. Again the supper may be made to fall within the patient's natural tolerance and the morning dose carry a fairly large breakfast and noon-day meal.

In our earlier work, after estimating the total number of grams of assistance needed for the whole day, we gave one-third of this amount before each meal. We soon found that it was impossible to keep the patient continuously "sugar-free" during the entire twenty-four hours without causing him to be hypoglycemic either in the afternoon or in the evening. By examining two hour specimens of urine during the day-time and single specimens as passed during the night, we found that if any sugar were passed in the urine it was passed in the morning. We then divided the total daily assistance into two parts and gave one-half of this amount before breakfast and one-half before supper. Again, we found it impossible to keep patients continuously "sugar-free." And again, on examining single specimens of urine, we found that the sugar was passed during the day and not during the night. It therefore appeared that the morning dose should be larger than the evening dose. This was logical, since the morning dose would care for portions, at least, of the morning and noon meals. We then planned to increase gradually the morning dose until the desired ratio was found. When  $\frac{4}{7}$  of the total dose were given before breakfast and  $\frac{3}{7}$  before supper, there was less sugar passed during the day time, but the patients often became hypoglycemic at night. When  $\frac{5}{8}$  of the



total dose were given before breakfast and  $\frac{3}{8}$  before supper, we were able to keep such patients continuously "sugar-free" without the appearance of symptoms of overdosage. In a few patients, we have given  $\frac{6}{9}$  of the total dose before breakfast and  $\frac{3}{9}$  before supper. Such patients became hypoglycemic in the day time and would pass sugar after supper. In the past six months, we have used this 5:3 ratio in the adjustment of the dose with remarkable success.

In the tabulated series, there are two patients, Nos. 6 and 19, whom we have been unable to keep continuously "sugar-free" without causing symptoms of marked overdosage. In these patients, whose tolerances were originally zero and in whom we noted no gain in tolerance for many months, we have given three doses per day in order to avoid the passage of too large amounts of sugar which would upset the glucose, fatty acid ratio. One comparatively large dose is given before breakfast and two smaller ones are given, one after the noon-day meal and one at bed-time. With this distribution, these patients pass very little sugar and are occasionally "sugar-free" throughout the twenty-four hours without any symptoms of overdosage. The tolerances in these patients are now growing and we hope in the near future by the three dose plan to be able to keep them continuously "sugar-free." Both of these patients have shown notable gains in weight and strength. Each has been changed from a bed-ridden invalid to an individual possessing the full enjoyment of a strenuous out-of-door life. One of them, aged thirteen, has gained in weight from 40 to 82 pounds; the other, aged twenty-five, from 61 to 119 pounds. We have not used a midnight dose lest the disturbance of rest would offset the good thereby gained.

In a number of patients, we have been able to give considerably more insulin than the calculated amount necessary to make up the difference between the natural tolerance and the sugar-formers of the diet, without producing any symptoms of overdosage. In one instance, in a case that afterward proved to be renal glycosuria, and before this was demonstrated, by gradually increasing the dose, we gave 90 grams of insulin assistance on a diet "G" of 92 before the patient had any symptoms of hypoglycemia. We often purposely give maximum doses of insulin in the hope that the pancreas may be given maximum rest. In these instances, the difference between the insulin "G" and the diet "G"



obviously does not represent the patient's real tolerance. To determine his tolerance, we gradually reduce the insulin until a small amount of sugar, one or two grams, appears in the urine and then increase the insulin until the patient is again "sugar-free" to the subnormal test explained below.

### *The Symptoms and Treatment of Overdosage With Insulin.*

When a rabbit is given an excessive dose of insulin, the blood sugar rapidly falls. When it reaches approximately 0.04%, convulsions usually occur which are promptly relieved by the administration of glucose. Patients may also become hypoglycemic from an overdosage with insulin. This may occur from unfamiliarity with the drug. It occurred with us more frequently in the past when the strength of the insulin was not as well standardized as it is now, especially, as with improved methods of preparation, we were making more potent extracts. It may occur if a patient's tolerance is unknown and if his diet is not carefully estimated, when it would be impossible to accurately adjust the dose, even if the value of the insulin were known. Overdosage with insulin excusably occurs in the first few weeks of treatment, when, by keeping the patient continuously free from acidosis and urinary sugar, his tolerance usually grows very rapidly. Under these conditions, the symptoms of overdosage are generally mild and easily treated if the patient is taught to recognize them early.

### *The Symptoms of Overdosage With Insulin.*

1. Hunger: As a patient's blood sugar falls, he usually experiences a keen appetite. This is not a very reliable symptom, because nearly all diabetic patients are notably hungry.
2. Slow mentality: A patient complains that he cannot think well or concentrate on any one thing.
3. Extreme weakness: This is probably the most reliable, early symptom. When a patient is metabolizing an ample diet, he should feel well and strong, but as he is overdosed with insulin, a feeling of weakness comes on. Sometimes patients describe this as a dizzy sensation.
4. Rapid pulse and respiration: The pulse is usually weak and accelerated and the respirations are rapid.

5. Visual disturbances: The patient complains of an inability to read due to double vision or the blurring of the print. He cannot see to write well. The eyes may ache and dark spots appear before them. Frequently these visual disturbances are the first symptoms observed by a new patient.

6. Shaky feeling: The expressions "shaky" and "the shakes" have been coined by the patients to describe their sensations, and although not scientific, are certainly well chosen. The patient simply shakes all over. He is not cold, nor are these symptoms associated with or followed by appreciable elevations in body temperature. We believe that this symptom is nature's method of causing the glycogen which has been stored in the liver and muscles, to be changed into glucose and poured into the blood stream.

7. Sweating: The "shaky" feeling is followed by a profuse sweat. This must be watched for, especially in new patients, as it is a very reliable symptom.

8. Unconsciousness: If a patient has been too seriously overdosed he may become unconscious, falling into a deep sleep from which it is impossible to rouse him until sugar has been administered. At this stage, he cannot be forced to swallow fluids.

9. Convulsions: If the overdose has been still greater, convulsions may occur. Fortunately these are very rarely seen in patients, but are of common occurrence in rabbits. In our experimental evaluation of insulin, we have used thousands of rabbits, and, in thousands of them, such convulsions have been produced; but, despite such marked overdosage, with the administration of glucose only a few rabbits have died in the entire series. The intravenous injection of 5 cc. of a 20% solution of glucose restores such convulsed rabbits in two or three minutes.

#### *The Treatment of Overdosage With Insulin.*

The treatment of the overdosage with insulin is probably started by nature as she changes the glycogen stores into sugar, and pours them into the blood stream. In this clinic, whenever a patient experiences the slightest symptoms of hypoglycemia, twenty grams of milk chocolate, having a food value of carbohydrate 10, protein 2 and fat 7, are given at once, followed by or dissolved in hot water. No attempt is made to determine whether

the urine or blood is too free from sugar, for we much prefer to give this emergency ration many times when it is not needed than to omit it once when it is needed. The milder symptoms, such as hunger, are promptly relieved by serving the tray a little earlier. The insulin treated patient is instructed never to be without his emergency, chocolate ration. Many other substances containing sugar-formers could be used and are used in lieu of chocolate. At first, we used white crackers, but, in these, the starch must be changed into sugar before it can be absorbed. Later, we used whole milk, but half pints of whole milk do not stay sweet any appreciable length of time, and are difficult for the patient to carry with him. Orange juice, when available, is an excellent form of a rapidly assimilable carbohydrate. Adrenalin may be administered hypodermically, which causes the body rapidly to change glycogen into glucose. In our experience, the milk chocolate, especially when followed by or dissolved in hot water, has proven to be an excellent form of medication. It is also very acceptable to the diabetic patient who for so many years has been without candy. Patients often welcome too sugar-free symptoms because of the chocolate candy antidote and will nibble away at the chocolate to make it last as long as possible when they should eat it as rapidly as possible.

When a patient cannot swallow, glucose should be given by vein at once. We keep on the hospital floors a number of ounce bottles of sterilized 50% glucose, and a 25 cc. syringe ready for instant use, although we are rarely obliged to resort to this form of medication. We have had no fatalities from an overdosage with insulin.

#### *The Education of the Patient.*

As in the past, the education of the patient is the most essential, and often-times the most difficult part of the treatment. We routinely give a series of eighteen formal lectures, covering a period of three weeks. The lectures, including the medical phases of the subject, are given by a physician, while the lectures covering the preparation of and calculation of the diet are given by the dietitian in charge. Special conferences are arranged for patients who, for some reason or other, are unable to keep up with the classes. It is often necessary to teach patients some elementary school arithmetic before they can make the necessary percentage calculations.

*The Testing of Urine for Sugar.*

In our experience, the teaching of patients to test their own urine for sugar has proven a more difficult problem than one would suppose. The following instructions to patients may not be out of place here.

The testing of the urine for sugar is difficult because all normal urine contains small amounts of sugar. Many testing solutions have been devised. Of the older solutions, Trommer's and Fehling's are perhaps the best known. At present, Benedict's and Haines' solutions are probably the most widely used in this country. Most of these solutions contain copper sulphate. When sugar (glucose) is added to an alkaline solution of copper sulphate and heated, the blue, soluble, copper sulphate is reduced to the insoluble, yellow, reddish brown or red oxide of copper. (If a substance is soluble, a clear, transparent solution is formed. One can see through Benedict's copper sulphate solution. If a substance is insoluble, the solution is turbid. When the soluble copper sulphate is reduced to the insoluble oxide of copper by sugar, the solution becomes turbid and cannot be seen through.) We have found Benedict's solution the most reliable of all the now used copper solutions. The test is made on a twenty-four hour specimen of urine as follows: 2.5 cc. of Benedict's solution are placed in a test tube. Four drops of urine are added. The test tube is then placed in a can of boiling water for six minutes. It may be heated over a flame to the boiling point and then boiled for one and one-half minutes. This method is not so satisfactory because of the danger of breaking the test tube, having the test tube boil over, or burning one's fingers. If sugar is present, in abnormal amounts, the insoluble, yellow, reddish brown or red oxide of copper is present. If there is only a small amount of sugar, and hence only a small amount of the yellow oxide of copper is formed, Benedict's solution will be changed to a turbid green. The green color is due to the mixing of the two colors, green and yellow. If more sugar is present, more of the yellow oxide of copper is formed and the solution will turn a turbid yellow-green. If still more sugar is present, and all of the blue copper sulphate is reduced, then the solution will either be a turbid yellow, reddish brown or red. On standing, the insoluble oxide of copper may settle out at the bottom of the test tube, leaving a clear, colorless solution above.

As stated above, all normal urines contain a small amount of sugar, but the urinary testing solutions, if used correctly, are not delicate enough to detect this small amount of sugar. By the use of blood sugar methods, the exact amount of reducing substances in normal urines has been accurately determined. Many twenty-four hour specimens of urine taken from twenty-six normal individuals were studied by Neuwirth.<sup>5</sup> The total sugar output for twenty-four hours varied from 0.614 to 1.386 grams, the average being 0.94 grams. Similar results were obtained by Kast, Croll, and Meyers<sup>6</sup> in the study of one hundred fifty-two aglycosuric, hospital patients. It will be sufficiently accurate, therefore, to say that the average



amount of sugar passed by a healthy individual is in the neighborhood of one gram in twenty-four hours.

Benedict's solution, and practically all other sugar testing solutions, do not show the presence of less than 0.05 per cent. sugar. One of the most common sources of error, in testing the urine for sugar, is a too concentrated urine. Let us suppose that a patient passes but 1000 cc. of urine in the twenty-four hours. The urine will contain approximately 1 gram of sugar, or 0.10 per cent. If this urine is tested as outlined above, a turbid green will usually appear, for Benedict's solution generally gives a positive test for sugar if the percentage exceeds 0.05 per cent. The entire twenty-four hour specimen of urine should total 2000 cc. One gram of sugar in 2000 cc. of urine is equal to 0.05 per cent. which is not detected in the urine when tested with Benedict's solution.

Another source of error comes from using too small drops. One official drop, or minim, is equivalent to approximately  $\frac{1}{16}$  cc. A comparatively small drop falls from a fine pointed medicine dropper, and normal sized drops fall from a blunt pointed medicine dropper. The blunt pointed medicine dropper should be used. After a medicine dropper has been filled with urine, it should be held horizontally and drops of maximum size allowed to accumulate and fall by their own weight from the blunt tip. The correct amount of Benedict's solution should be used. We have seen patients use one cc. of the blue solution and four cc. of urine, which procedure always gives a positive test for sugar, as it should.

By taking two hour samples of blood throughout the day, we have found that when insulin is being used it is possible to maintain the blood sugar of most patients at a subnormal level through the greater portion of the twenty-four hours. It is not always possible in patients whose natural tolerances are too low to maintain a bed-rest diet to secure a normal blood sugar before breakfast without giving a dose of insulin late at night. But even in these patients, blood sugars of 0.08 and 0.09 per cent. are often seen during the day without the patient becoming hypoglycemic. The details of this work will be published at a later date. We have found it entirely feasible to maintain insulin patients negative to subnormal amounts of sugar in the twenty-four hour urine, and hence believe that the following test for subnormal amounts of sugar in the urine will be useful to patients. When the urines are negative to the subnormal test, the blood sugars throughout the day average lower than those of normal individuals.

#### *The Test for Subnormal Amounts of Sugar in the Urine.*

We now believe that when small amounts of sugar are passed in the urine, the quantity of sugar passed is proportional to the blood sugar. As the blood sugar rises, increasing amounts are passed in the urine. When the blood sugar is normal or slightly



subnormal, as it usually is when sufficient insulin is being used, subnormal amounts of sugar are passed in the urine. We believe that we have devised a test whereby from a study of the urine we can predict whether the blood sugar will average high, normal or subnormal.

The test is a simple one, for it only requires double the amount of urine used in the normal test. Sometimes patients pass too small amounts of urine; then proportionately fewer drops of urine should be used to make the test. Again, patients may pass more than the normal amount of urine; then more than the ordinary number of drops should be used, lest from the dilution of the urine abnormal amounts of sugar may escape detection. The following table has been devised to take into consideration all of these factors. Patients not taking insulin may not be able to keep their urines negative to the subnormal test, but patients taking insulin should attempt to keep their urine free to the subnormal test.

24 Hour Urine	Normal Test	Subnormal Test
500 cc. ....	1 drop .....	2 drops
750 cc. ....	1 drop .....	3 drops
1000 cc. ....	2 drops.....	4 drops
1250 cc. ....	2 drops.....	5 drops
1500 cc. ....	3 drops.....	6 drops
1750 cc. ....	3 drops.....	7 drops
2000 cc. ....	4 drops.....	8 drops
2250 cc. ....	4 drops.....	9 drops
2500 cc. ....	5 drops.....	10 drops
2750 cc. ....	5 drops.....	11 drops
3000 cc. ....	6 drops.....	12 drops
3250 cc. ....	6 drops.....	13 drops
3500 cc. ....	7 drops.....	14 drops
3750 cc. ....	7 drops.....	15 drops
4000 cc. ....	8 drops.....	16 drops

The patient should aim to drink sufficient fluid to keep the urine volume in the neighborhood of 2000 cc., when 4 drops of urine are the correct amount to use for the normal test, and 8 drops for the subnormal test with 2.5 cc. of Benedict's solution.

#### *Routine Diets Used.*

The following diets are used routinely, but are modified somewhat to meet the needs of each individual case.

#### The severe acidosis diet.

Type of Food	B.	D.	S.	Grams	C.	P.	F.
Orange Juice .....	(small portions)			1000	170	20	0
Dry Cereal .....	30	30	30	90	61	14	6
Skimmed Milk .....	100	100	100	300	15	9	3
Total Calories 1237. "G"—272.					246	43	9

## 1000 Calory Diet.

Type of Food	B.	D.	S.	Grams	C.	P.	F.
3% Vegetables .....	266	266	266	800	24	8	0
Eggs .....	1			(1)		6	6
Lean Meat (15% Fat)....		25	25	50		13	8
Butter .....	5	10	10	25			21
Bran Muffins .....	1	1	1	(3)	9	9	18
40% Cream .....	25	25	25	75	2	2	30
					35	38	83

Total Calories 1039. "G"—65.

## 1500 Calory Diet.

Type of Food	B.	D.	S.	Grams	C.	P.	F.
3% Vegetables .....	233	233	233	700	21	7	0
6% Vegetables .....		100	100	200	12	2	0
Eggs .....	1			(1)		6	6
Bacon .....	10			10		2	4
Lean Meat (15% Fat)....		25	25	50		13	8
Butter .....	5	10	10	25			21
Bran Muffins .....	1	1	1	(3)	9	9	18
Dry Cereal .....	10			10	8	1	1
40% Cream .....	50	50	50	150	5	3	60
					55	43	118

Total Calories 1454. "G"—92.

## 2000 Calory Diet.

(Low protein, used in cases of high blood pressure.)

Type of Food	B.	D.	S.	Grams	C.	P.	F.
3% Vegetables .....	100	200	200	500	15	5	0
6% Vegetables .....		100	100	200	12	2	0
9% Vegetables .....		50	50	100	9	1	0
Eggs .....	1			(1)		6	6
Bacon .....	10			10		2	4
Lean Meat (15% Fat)....		25	25	50		13	8
Butter .....	20	20	20	60			51
Bran Muffins .....	1	1	1	(3)	9	9	18
40% Cream .....	66	66	66	200	6	4	80
10% Fruits .....	33	33	33	100	10	1	0
Dry Cereal .....	20			20	16	2	2
					77	45	169

Total Calories 2009. "G"—120.

## 2000 Calory Standard Diet.

Type of Food	B.	D.	S.	Grams	C.	P.	F.
3% Vegetables .....	100	300	300	700	21	7	0
6% Vegetables .....		125	125	250	15	3	0
Eggs .....	2			(2)		12	12
Bacon .....	10			10		2	4
Lean Meat (15% Fat)....		50	50	100		25	15
Butter .....	10	15	15	40			34
Bran Muffins .....	1	1	1	(3)	9	9	18
40% Cream .....	66	66	66	200	6	4	80
Dry Cereal .....	20			20	16	2	2
					67	64	165

Total Calories 2009. "G"—120.

## 2200 Calory Diet.

Type of Food	B.	D.	S.	Grams	C.	P.	F.
3% Vegetables .....	100	300	300	700	21	7	0
6% Vegetables .....		125	125	250	15	3	0
Eggs .....	2			(2)		12	12
Bacon .....	10			10		2	4
Lean Meat (15% Fat)....		50	50	100		25	15
Butter .....	20	20	20	60			51
Bran Muffins .....	1	1	1	(3)	9	9	18
40% Cream .....	66	66	66	200	6	4	80
10% Fruits .....	33	33	33	100	10	1	0
Dry Cereal .....	20			20	16	2	2
					77	65	182

Total Calories 2206. "G"—133.

## 2500 Calory Diet.

Type of Food	B.	D.	S.	Grams	C.	P.	F.
3% Vegetables .....	100	300	300	700	21	7	0
6% Vegetables .....		100	100	200	12	2	0
9% Vegetables .....		50	50	100	9	1	0
Eggs .....	2			(2)		12	12
Bacon .....	10			10		2	4
Lean Meat (15% Fat)....		50	50	100		25	15
Butter .....	15	15	15	45			38
Bran Muffins .....	1	1	1	(3)	9	9	18
40% Cream .....	100	100	100	300	9	6	120
10% Fruits .....	33	33	33	100	10	1	
Dry Cereal .....	20			20	16	2	2
					86	67	209

Total Calories 2493. "G"—146.

## 2600 Calory Diet, with Milk.

Type of Food	B.	D.	S.	Grams	C.	P.	F.
3% Vegetables .....		200	200	400	12	4	0
6% Vegetables .....		100	100	200	12	2	0
Eggs .....	2			(2)		12	12
Bacon .....	10			10		2	4
Lean Meat (15% Fat)....		50	50	100		25	15
Butter .....	15	15	15	45			38
Bran Muffins .....	1	1	1	(3)	9	9	18
40% Cream .....	88	88	88	265	8	6	106
10% Fruits .....	33	33	33	100	10	1	
Dry Cereal .....	15			15	12	2	2
Whole Milk .....	200	200	200	600	30	18	24
					93	81	219

Total Calories 2667. "G"—162.

## 3000 Calory High Fat Diet.

Type of Food	B.	D.	S.	Grams	C.	P.	F.
3% Vegetables .....		200	200	400	12	4	0
6% Vegetables .....		100	100	200	12	2	0
Olive Oil .....		32	32	64			64
Eggs .....	2			(2)		12	12
Bacon .....	10			10		2	4
Lean Meat (15% Fat)....		50	50	100		25	15
Butter .....	15	15	15	45			38
Bran Muffins .....	1	1	1	(3)	9	9	18
40% Cream .....	66	66	66	200	6	4	80
10% Fruit .....	33	33	33	100	10	1	
Dry Cereal .....	15			15	12	2	2
Whole Milk .....	200	200	200	600	30	18	24
					91	79	257

Total Calories 2993. "G"—163.

In the calculation of these diets the following simple table has been used. Fractions are purposely omitted. Five or more tenths of a gram are reckoned as an additional gram and less than five-tenths of a gram are ignored.

## APPROXIMATE FOOD VALUE (100 GRAM PORTION)

	Carbo- hydrate	Protein	Fat
3% Vegetables (weighed fresh or after cooking).....	3	1	0
Lettuce			
Asparagus			
Celery			
Swiss Chard			
Mushrooms			
Chioté			
Cucumbers			
Rhubarb			
Sauerkraut			
Tomatoes			
Pumpkin			
Spinach			
Beet Greens			
Brussels Sprouts			
Summer Squash			
Cauliflower Stalk			
6% Vegetables .....	6	1	0
Water Cress			
Egg Plant			
Turnips			
Banana Squash			
Okra			
Cabbage			
Artichoke			
Cauliflower			
Radishes			
String Beans			
9% Vegetables .....	9	1	0
Celery Root			
Rutabagas			
Carrots			
Kohlrabi			
Hubbard Squash			
Onions			
Oyster Plant			
Beets			
15% Vegetables .....	15	2	0
Parsnips			
20% Vegetables .....	20	2	0
Potatoes			
Green Corn			



10% Fruits .....	10	1	0
Strawberries			
Oranges			
Muskmelons			
Cranberries			
Raspberries			
Grapefruit			
Watermelons			
Guavas			
Peaches			
Lemons			
Blackberries			
Pineapple			
Eggs (each) .....	0	6	6
Bacon, 10 gram portion .....	0	2	4
Meat, lean, cooked .....	0	25	15
Ham, lean, cooked .....	0	20	20
Butter .....	0	0	85
Cream, 20% Butter Fat .....	4	3	20
Cream, 40% Butter Fat .....	3	2	40
Whole Milk .....	5	3	4
Skimmed Milk .....	5	3	1
Unwashed Bran Muffin .....	3	3	6
Dry Cereal (10 gram portion) .....	8	1	1
Bread .....	53	9	2
Bran (Baker's) .....	27	10	5

## RECIPES FOR DIABETIC DIETS.

## Unwashed Bran Muffins.

Unwashed Bran .....	66 gms.	Butter .....	25 gms.
Soda .....	$\frac{1}{8}$ tsp.	Water .....	$\frac{1}{3}$ cup
Salt .....	$\frac{1}{4}$ tsp.	Eggs .....	2
Baking Powder .....	1 tsp.		

Mix the dry ingredients. Add the melted butter and water. Add egg yolks, well beaten, and fold in the stiffly beaten whites.

	C.	P.	F.
This quantity makes 6 Muffins. Food value	3	3	6

## Mayonnaise Dressing.

Olive Oil .....	480 gms.	Cayenne Pepper .....	$\frac{1}{8}$ tsp.
Vinegar .....	35 gms.	Salt .....	1 tsp.
Egg yolk .....	1	Vinegar .....	35 gms.

Beat the egg yolk. Then add small amount of oil and vinegar alternately until the mixture is quite stiff. The remainder of the oil and vinegar may be added in larger quantities without harm. Add the salt and cayenne last. Beat thoroughly. Place the dressing in a jar and keep in a cool place.

100 grams of dressing equals 90 grams of fat.

## Diabetic Salad Dressing.

Mineral Oil .....	1½ C.	Cayenne Pepper .....	¼ tsp.
Egg .....	1	Salt .....	½ tsp.
Vinegar .....	2	tbsp.	

Beat the egg well. Then add small amount of oil and vinegar, alternately until the mixture is quite stiff. The remainder of the oil and vinegar may be added in larger quantities without harm. Add the salt and cayenne last. Beat thoroughly. Place the dressing in a jar and keep in a cool place. Practically no food value.

## Oatmeal.

Oatmeal, 120 gms. to 1 qt. of water.

Boil 5 minutes, then steam three hours. 10 gms. dry weight equals 70 gms. cooked oatmeal.

Food value; Carb. 8; Prot. 1; Fat 1.

## DISCUSSION.

In this series, there are five coma cases. Three were seen after they had developed deep coma and these three died, although large amounts of insulin and sugar were given intravenously and otherwise. It would appear that even with insulin, patients who have been in deep coma for some time, have a fatal prognosis, probably due to irreparable cell changes. Case No. 5 had been vomiting for forty-eight hours and does not remember having been given insulin and oatmeal gruel, although two hours later she was fully conscious. Case No. 66 was unable to swallow and had a severe acidosis and the deep breathing typical of coma. Within twenty minutes after the administration of two cc. of insulin, worth twenty-seven grams of sugar-burning power per cc., she was able to drink orange juice freely.

The lowest possible bed-rest maintenance diet of an adult is probably about 1000 calories, which requires a diet "G" in the neighborhood of 65 grams. In this series, there were sixty cases whose tolerances were 65 or less. Probably very few of these patients could have been rendered "sugar-free" and kept continuously so, even if kept constantly in bed.

In this series, there are three patients, Nos. 10, 14 and 62, in whom the diabetes is complicated by extremely severe tuberculosis. Patient No. 10, whose tolerance was 55 "G," whose sputum was very copious and contained innumerable acid-fast bacilli, and whose lungs had developed marked cavities, has gained fifty-one

pounds in weight. He is now free from sputum, and the lung condition shows healing by fibrosis. Patient No. 14, having a tolerance of 66 "G" and a similar lung condition, has gained twenty-four pounds. The sputum is very much reduced and the lungs show a similar healing process, although not to such a marked degree. Patient No. 62, with a tolerance of 17, probably has, in addition to her pulmonary tuberculosis with cavity formation, a disseminated tuberculosis. Her urine contains considerable albumin and many acid-fast bacilli. She has gained only 4.8 pounds in weight, but her temperature has subsided and the lungs show some healing by fibrosis. We consider her prognosis very unfavorable.

In this series, twelve surgical operations have been performed with no complications. Patients Nos. 3, 8, 32, 41, 73, 87 and 88 have had their tonsils removed. Patient No. 80 had a large colloid goitre removed under ether anaesthesia. This goitre was unsightly and so large as to give the patient a wry-neck appearance. It was removed because of pressure symptoms on the trachea and oesophagus. Patient No. 21 has recently undergone an appendectomy with no complications.

In this series, there are seventy-seven patients taking insulin at the present time. The average daily amount taken by them interpreted in iletin units amounts to 54 units on a diet average of 2338 calories. The average growth in tolerance in this group of 77 cases has amounted to 31.2 grams of sugar formers, insofar as can be determined by the methods outlined above.

The average gain in weight in patients who have had insulin for three months or more has amounted to 3.8 pounds per patient per month. The maximum gain in weight, in any one patient, has been fifty-nine pounds.

In this series, there are four patients whose tolerances are sufficiently high to warrant discontinuing insulin treatment.

We have had no anaphylactic reactions from the use of either insulin or iletin. There has been a moderate amount of induration from some of the iletin used. All patients have been remarkably free from hypodermic difficulties. In the beginning, we had two instances of small subcutaneous abscesses developing from the administration of insulin into the buttocks, and hence we do not advise this locus for the injections. One patient, No. 10, has taken 750 injections in one small area of the left arm with no

untoward effects. This patient claims, as do many others, that the hypodermic injections are painless.

Thoroughly trained patients apparently have little difficulty in administering their own insulin, and in managing their diets at home. The rewards of an ample diet and the increased weight and strength so far outweigh the penalties of the hypodermic injections, that patients enthusiastically take their insulin. Many of the children have learned to take their own hypodermic injections.

There are a few patients in this series, Nos. 5, 11, 34, 48, 56, 61, 75, 77 and 80, who have refused for various reasons to continue taking insulin. Patient No. 5 is a frail lady suffering from carcinoma of the breast, but who does very well on a 949 calorie diet. Patient No. 34, an old man 71 years of age, left the hospital seven days after admission when he realized that insulin was not an absolute cure. Patient No. 48 said that he could not afford insulin, and we lost track of him when he returned to his home in Colorado. Patient No. 56 claimed that he had had previous excellent results from a certain pancreatic compound. He promptly passed sugar in the urine when he discontinued insulin and resumed the compound. Patient No. 75 was mentally deficient and remained in the hospital only two days.

In this series, there are five patients who have died from causes other than their diabetes. Patient No. 2 died from bronchopneumonia. Patient No. 7 was admitted with precomatose symptoms which we were able to alleviate with insulin, but she died from an acute heart weakness. No. 36 died of chronic myocarditis with decompensation of long standing. No. 57 was a morphine addict who committed suicide after leaving the hospital. No. 69 was a morphine addict who died following a diet spree and influenza.

### CONCLUSIONS.

1. With the exception of a few cases that were seen after the onset of deep coma, insulin has proven a specific in the relief of all diabetic symptoms. Patients promptly become free from acidosis and from abnormal sugar in the urine with the loss of thirst and frequent urination. As their diets are increased, they gain in weight and strength. Many of the patients, who were emaciated and weak to an extreme degree, and who, before insulin

treatment, had a fatal prognosis, have already regained their normal weight, health and strength.

2. Insulin is not a cure for diabetes and patients will need to exercise even greater care than ever before with their diets, for on the one hand their diabetic symptoms may return if their diets exceed their natural tolerances and their insulin, or, on the other hand, they may become hypoglycemic from overdosage.

3. In addition to enabling patients to metabolize more food, growths in natural tolerance have been observed in almost every patient who has been treated for a sufficient length of time. In one instance, such a growth did not occur until the patient had been under treatment for nearly one year. Growths of tolerance were obtained in the past by dietetic management, but since in the terminal stages of the disease patients often cannot be kept continuously "sugar-free" and free from acidosis, even if kept constantly in bed, in them opportunities for tolerance growth did not occur. Better growths in tolerance are observed in diet plus insulin treated patients than in patients under dietetic management alone. Previously mismanaged cases have shown the most marked growths in tolerance.

4. We have had no success with any method of administration other than the hypodermic route. We have given, orally, twenty-five times the subcutaneous dose to patients and to experimental animals with no success whatever. Methods of oral administration should, however, be worked out if it is possible to circumvent the action of the alimentary, digestive enzymes.

5. We believe that insulin has a definite sugar-metabolizing power which does not vary appreciably in patients of different degrees of severity, does not vary with age and weight or with low and high diets. The Eli Lilly unit, as used by us, has a sugar metabolizing power of approximately 0.85 grams per unit or 17 grams per cc. of their H-20 product, and 34 grams per cc. of their H-40 product. Their unit has a very constant value.



## REFERENCES.

1. Banting, F. G., Best, C. H. The Internal Secretion of the Pancreas, *The Journ. of Lab. and Clin. Med.*, 7, 1922, 251.  
Banting, F. G., Best, C. H. Pancreatic Extracts. *The Journ. of Lab. and Clin. Med.*, 7, 1922, 464.  
Banting, F. G., Best, C. H. Pancreatic Extracts in the Treatment of Diabetes, Preliminary Report. *Canadian Med. Assn. Journ.*, 12, 1922, 141.
2. Banting, F. G., Best, C. H., Collip, J. B., MacLeod, J. J. R. Preliminary Studies of the Physiological Effects of Insulin. *The Transactions of the Royal Society of Canada*, 1922, 16.
3. *Journ. A. M. A.*, Vol. 80, No. 22, p. 1617.
4. Woodyatt, R. T. Objects and Methods of Diet Adjustment in Diabetes. *Archives of Internal Medicine*, Vol. 28, No. 2, p. 125.
5. Neuwirth, Isaac. A Study of Urinary Sugar Excretion in 26 Individuals. *Journ. of Biol. Chem.*, Vol. LI, 1, p. 11.
6. Kast, Ludvig; Croll, Hilda; Meyers, Victor C. The Excretion of Sugar in the Urine in Health and Disease. *Journ. of Lab. and Clin. Med.*, Vol. VIII, 4, pp. 227-241.

CHART No. 1

[illegible]

CHART No. 1—Continued

No.	Sex	Age	Duration Yrs.	Date	Net Wt. lbs.	Gain in Wt.	DIET				Insulin "G."	Toler- ance "G."	Ile- tin Units	Urine Sugar		Blood Sugar %	Acidosis	
							C.	P.	F.	Cal.	"G."			%	Grams		Acetone	NH <sub>3</sub>
8	F	9	2	1-8-22	56.0	.....	?	?	?	?	?	?	.....	.....	.....	.....	+++	.....
				9-28-22	58.0	.....	49	58	116	1472	94	0	.....	2.6	.....	.....	0	.....
				4-10-23	60.0	.....	?	?	?	?	?	0	.....	.....	.....	0.10	0	.....
				4-28-23	65.0	.....	53	40	118	1434	88	30	.....	1.5	.....	.....	++	.....
				6-18-23	79.0	21.0	83	57	191	2279	135	24	.....	0	.....	.....	0	.....
9	F	53	10	8-17-22	84.0	.....	20	57	92	1136	62	0	.....	0	.....	.....	0	.....
				7-15-23	123.0	39.0	77	65	182	2206	133	68	.....	0	.....	.....	0	.....
10	M	30	10	8-5-22	102.5	.....	?	?	?	?	?	0	.....	0	.....	.....	0	.....
				7-15-23	154.0	51.5	86	67	273	3069	152	96	.....	0	.....	.....	0	.....
11	M	60	2	9-15-22	?	.....	55	44	129	1557	93	0	.....	1.5	27.0	.....	+++	2.04 Gm
				10-7-22	?	.....	55	44	129	1557	93	0	.....	0	.....	.....	0	.....
12	M	49	4	9-26-22	109.0	.....	49	55	122	1514	92	0	.....	0	.....	0.18	0	.....
				7-15-23	134.0	25.0	82	68	211	2499	142	42	.....	0	.....	0	0	.....
13	M	31	5	9-29-22	130.0	.....	70	40	124	1396	65	0	.....	0	.....	.....	0	.....
				7-10-23	157.0	27.0	72	93	263	3063	152	74	.....	0	.....	.....	0	.....
14	M	33	4	9-30-22	115.0	.....	55	42	125	1513	63	0	.....	0	.....	0.18	0	.....
				7-15-23	139.0	24.0	91	79	257	2993	162	96	.....	0	.....	0	0	.....
15	M	10	4	10-8-22	58.0	.....	?	?	?	?	?	?	.....	5.1	.....	.....	+++	.....
				11-8-22	59.0	.....	55	43	118	1462	92	60	.....	0	.....	.....	0	.....
				7-15-23	81.0	23.0	80	55	179	2151	130	69	.....	0	.....	.....	0	.....

In this case insulin was given for 7 days to control the acidosis.

The patient was readmitted April 10, 1923, after a series of acute respiratory infections.

In this patient there is a complication of extremely severe tuberculosis.

This patient was a surgical case and was given insulin for four days only.

In this patient there is a complication of extremely severe tuberculosis.

CHART No. 1—Continued

No.	Sex	Age	Duration Yrs.	Date	Net Wt. lbs.	Gain in Wt.	DIET					Insulin "G"	Toler- ance "G"	Ile- tin Units	Urine Sugar		Blood Sugar %	Acidosis	
							C.	P.	F.	Cal.	"G"				%	Grams		Acetone	NH <sub>3</sub>
16	M	25	8	10-11-22 6-15-23	134.0 162.0	28.0	51 114	47 93	178 276	1994 3312	96 195	0 63	96 132	---	0 0	---	---	0 0	---
17	F	35	1.5	10-23-22 6-7-23	94.8 108.0	13.2	54 75	46 67	118 183	1462 2215	93 132	0 30	93 100	---	0 0	---	---	0 0	---
18	F	13	4	10-24-22 7-15-23	40.0 82.5	42.5	?	?	?	1265 1790	126 146	0 64	0 40	---	---	130.0 25.0	---	+++ 0	---
This is the second patient in the entire series in whom it has seemed inadvisable to keep the urine continuously free from abnormal sugar.																			
19	M	29	1	10-29-22 11-8-22 6-14-23	106.0 108.0 130.0	---	?	?	?	1462 1813	92 120	28 50	64 70	---	5.1 0	---	---	+++ 0 0	---
20	F	12	3	10-30-22 11-8-22 3-23-23	60.0 59.0 73.0	---	?	?	?	1030 1680	65 100	44 38	21 62	---	2.2 0	---	---	+++ 0 0	---
21	M	36	2.5	10-31-22 11-8-22 6-12-23	111.0 121.0 147.0	---	?	?	?	1120 2958	73 154	55 35	18 119	---	2.6 0	---	---	+++ 0 0	---
22	M	23	3	11-1-22 11-8-22 4-1-23	89.0 90.0 104.0	15.0	?	?	?	1030 2206	65 133	40 72	25 61	---	4.7 0	---	---	+++ 0 0	---
23	F	17	1	11-2-22 11-9-22 6-19-23	132.0 132.0 147.0	---	?	?	?	1030 1402	65 143	60 21	5 122	---	3.3 0	---	---	+++ 0 0	---
24	F	27	1.3	11-3-22 11-8-22 7-15-23	101.6 103.2 120.0	18.4	?	?	?	1030 1690	65 75	60 30	5 45	---	3.1 .9	---	---	+++ +++ 0	---

Because of the cost of iletin this patient could afford only a low diet. With the assistance of our insulin subsidy fund she is now aglycosuric and on an adequate diet.

CHART No. 1—Continued

No.	Sex	Age	Duration Yrs.	Date	Net Wt. lbs.	Gain in Wt.	DIET				Insulin "G"	Toler- ance "G"	Ins- lin Units	Urine Sugar		Blood Sugar %	Acidosis	
							C.	P.	F.	Cal.	"G"			%	Grams		Acetone	NH <sub>3</sub>
25	F	39	8	11-4-22 11-8-22 3-25-23	122.4 123.3 140.0	17.6	? 54 77	? 46 45	? 118 169	? 1462 2009	? 92 120	? 44 40	?	†† 0 0	..... ..... .....	..... ..... .....	†† 0 0	..... ..... .....
26	F	37	3	11-4-22 11-8-22 6-9-23	82.5 84.5 122.0	39.5	? 35 77	? 38 45	? 82 169	? 1030 2009	? 65 120	? 60 51	?	5.1 0 0	..... ..... .....	..... ..... .....	†††† † 0	..... ..... .....
27	M	39	9	11-10-22 11-23-22 7-15-23	106.0 105.5 159.0	53.0	? 35 99	? 38 52	? 83 266	? 1039 2998	? 65 155	? 65 94	?	4.2 0 0	..... ..... .....	..... ..... .....	††† 0 0	..... ..... .....
28	F	49	?	11-10-22	?	.....	? ?	? ?	? ?	? ?	? ?	?	?	†††	.....	.....	coma	.58 Gm-L
This patient was admitted after having been in deep coma for 12 hours. Her systolic blood pressure was 50. Insulin and glucose intravenously failed to cause any response. She died 4 hours after entering the hospital.																		
29	F	31	1	11-12-22 11-21-22 6-16-23	84.0 93.0 109.0	25.0	? 35 55	? 38 43	? 83 118	? 1039 1454	? 65 92	? 38 20	?	1.3 tr 0	..... ..... .....	..... ..... .....	††† tr 0	..... ..... .....
30	F	58	2	11-10-22 11-20-22 6-11-23	109.6 111.0 124.0	14.4	? 35 70	? 38 65	? 83 189	? 1039 2241	? 65 127	? 30 36	?	2.5 0 0	..... ..... .....	..... ..... .....	††† 0 0	..... ..... .....
31	M	31	3	11-19-22 6-6-23	131.0 155.0	24.0	75 81	48 79	169 222	2013 2638	120 149	0 7	120 142	0 0	..... .....	..... .....	0 0	..... .....
32	M	9	3	11-19-22 11-30-22 6-19-23	53.2 54.7 61.0	7.8	? 35 67	? 38 64	? 83 165	? 1039 2009	? 65 120	? 15 19	?	5.1 0 0	..... ..... .....	..... ..... .....	†††† 0 0	2.4 Gm ..... .....
33	F	57	5	11-20-22 12-21-22	144.6 144.1	.....	? 35	? 38	? 83	? 1039	? 65	? 0	?	.2 0	..... .....	..... .....	0 0	..... .....

The patient used insulin for 6 months, and then remained sugar free on the following diet:



CHART No. 1—Continued

No.	Sex	Age	Duration Yrs.	Date	Net Wt. lbs.	Gain in Wt.	DIET				Insulin "G."	Tolerance "G."	Ile- tin Units	Urine Sugar		Blood Sugar %	Acidosis			
							C.	P.	F.	Cal.				"G."	%		Grams	Acetone	NH3	
34	M	73	0.5	6-15-23	146.0	2.0	67	64	165	2009	120	0	120	0	0	0	0			
				11-21-22	144.8		35	38	83	1039	65	0	?					+		
				11-25-22	144.3		35	38	83	1039	65	28	37	3.7	0		0			
This patient remained in the hospital only 7 days.																				
35	F	33	5	11-22-22	73.7		?	?	?	?	?	0	?		2.9		+++			
				12-2-22	78.2		35	38	83	1039	65	24	41	0		.08	0			
				6-11-23	123.0	49.3	81	81	221	2638	150	78	72	0			0			
36	M	54	3	11-24-22	102.0		?	?	?	?	?	0	?		+++		++			
				12-31-22	116.0	14.0	54	46	118	1462	92	48	44	0			0			
				This patient was admitted with an acutely decompensated heart, and died from cardiac failure, Jan. 17, 1923.																
37	M	18	0.5	11-25-22	121.5		?	?	?	?	?	0	?		.5		+++			
				11-27-22	121.7		35	38	83	1039	65	34	31	0		0				
				3-21-23	131.0	9.5	115	60	150	2050	165	105	60	0			0			
38	F	41	2	11-28-22	109.0		?	?	?	?	?	0	?		.2		+			
				12-14-22	112.5		54	46	118	1462	92	25	67	.1		0		0		
				1-15-23	112.5	3.5	77	45	169	2009	120	20	100	0			0		0	
39	F	41	1	10-28-22	95.0		?	?	?	?	?	0	?		.7		+++			
				11-7-22	97.7		75	48	169	2013	120	80	40	.1		0		0		
				7-15-23	128.2	28.2	77	65	182	2206	133	84	49	0			0		0	
40	M	39	4	12-4-22	115.2		?	?	?	?	?	0	?		.2		0			
				12-15-22	112.5		55	43	118	1454	92	27	65	0		.12	0		0	
				6-21-23	139.4	24.2	91	79	257	2993	163	98	65	0			0		0	
41	M	14	0.5	12-7-22	94.6		75	48	169	2023	120	0	120	0	0		0			
				6-10-23	116.0	21.4	86	67	209	2493	146	7	139	0		.10	0		0	

CHART No. 1—Continued

No.	Sex	Age	Dur- ation Yrs.	Date	Net Wt. lbs.	Gain in Wt.	DIET					Insulin "G."	Toler- ance "G."	Insulin Units	Urine Sugar		Blood Sugar %	Acidosis		
							C.	P.	F.	Cal.	"G."				%	Grams		Acetone	NH3	
42	M	40	1	12- 8-22 12-28-22 6-14-23	121.7 122.5 140.0	..... ..... 18.3	?	?	?	?	?	?	0 27 13	?	65 159	..... ..... .....	1.6 0 0	..... ..... .....	++++ 0 0	..... ..... .....
43	M	41	1.5	12-12-22 6- 9-23	106.7 124.0	..... 17.3	54 78	46 66	118 223	1462 2583	92 138	0 36	0 102	?	92 102	..... 42	0 0	.16 .....	0 0	..... .....
44	M	50	10	12-15-23	?	.....	?	?	?	?	?	0	?	?	+++	.....	.....	coma	.....	.....
This patient was seen after having been in deep coma for more than ten hours. Large doses of insulin and glucose were given with no response. He died 8 hours later.																				
45	M	19	5	12-15-22 12-22-22 6-12-23	96.0 92.5 130.0	..... ..... 34.0	?	?	?	?	?	?	0 35 42	?	30 91	..... ..... 50	1.4 .1 0	..... ..... .....	+++ 0 0	..... ..... .....
46	M	25	3	12-20-22 12-28-22 6- 8-23	99.8 99.7 120.0	..... ..... 20.2	?	?	?	?	?	?	0 10 79	?	55 60	..... ..... 93	7.2 0 0	..... ..... .....	++++ 0 0	..... ..... .....
47	M	20	0.5	12-25-22 6- 8-23	130.6 164.0	..... 33.4	35 77	38 63	83 169	1039 2081	65 131	0 17	0 65	?	114	..... 20	0 0	..... .....	0 0	..... .....
48	M	58	7	12-27-22 1-19-23	146.0 139.4	.....	?	?	?	?	?	?	0 69	?	23	.....	5.7 113.0 0	..... ..... .....	+	.....
This patient left the hospital without permission, and has since disregarded dietetic management.																				
49	M	24	4	12- 2-22 12-16-22 6-20-23	123.5 124.5 131.0	..... ..... 7.5	?	?	?	?	?	?	0 35 51	?	30 123	..... ..... 60	5.8 0 0	..... ..... .....	++ 0 0	..... ..... .11
50	M	36	1	1- 3-23 6- 9-23	130.0 139.5	..... 9.5	54 78	46 46	118 225	1446 2521	92 128	0 17	0 111	?	92 111	..... 20	0 0	..... .....	0 0	..... .....

CHART No. 1—Continued

No.	Sex	Age	Dur- ation Yrs.	Date	Net Wt. lbs.	Gain in Wt.	DIET				Insulin "G"	Toler- ance "G"	He- in Units	Urine Sugar		Blood Sugar %	Acidosis			
							C.	P.	F.	Cal.				"G"	%		Grams	Acetone	NH3	
51	M	40	1	12-29-23	167.0	.....	97	56	213	2529	150	0	150	0	.....	.14	0	.....		
Insulin given for one month only.																				
52	F	55	2	1-7-23	86.2	.....	?	?	?	?	?	0	?	.....	1.0	.....	.....	+++	.....	
				1-22-23	91.6	.....	55	43	118	1454	92	62	30	0	.....	0	.....	0	.....	
				7-15-23	109.0	13.8	77	65	182	2206	133	44	89	0	.....	0	.....	0	.....	
53	M	35	2	1-8-23	140.0	.....	28	66	110	1366	77	0	77	0	.....	.12	0	.....		
				7-15-23	163.0	23.0	84	72	240	2790	150	63	87	0	.....	0	.....	0	.....	
54	F	6	3	1-10-23	33.1	.....	?	?	?	?	?	0	?	.....	2.3	.....	.....	+++	.....	
				1-14-23	31.8	.....	35	41	85	1069	65	23	42	0	.....	0	.....	0	.....	
				6-9-23	34.0	0.9	35	38	78	994	65	4	61	0	.....	0	.....	0	.....	
55	F	50	3	1-10-23	108.1	.....	?	?	?	?	?	0	?	.....	6.9	.....	.....	+	.....	
				1-17-23	104.7	.....	55	43	118	1454	92	16	76	0	.....	.17	0	.....	0	.....
				6-14-23	144.0	35.9	77	65	182	2206	133	25	108	0	.....	.20	0	.....	0	.....
56	M	57	7	1-11-23	122.0	.....	?	?	?	?	?	0	?	.....	6.8	.....	.....	+	.....	
				1-29-23	124.7	.....	55	43	118	1454	92	60	32	0	.....	.....	0	.....	0	.....
				4-24-23	128.0	6.0	77	45	169	2009	120	0	?	.....	10.0	.....	0	.....	0	.....
This patient is now taking a proprietary pancreatic compound which he thought had benefited him in the past.																				
57	M	53	5	1-15-23	94.2	.....	?	?	?	?	?	0	?	.....	+++	.....	.....	+++	.....	
				2-6-23	90.6	.....	77	65	182	2206	133	40	93	0	.....	.....	.....	0	.....	
				3-21-23	94.8	.....	55	43	118	1454	92	26	66	.2	.....	.....	0	.....	0	.....
This patient was a morphine addict taking as high as 50 grains a day. Over a period of 12 weeks we were able to reduce his morphine to 12 grains a day. Friends supplied him with additional drug; he lost his morale and soon after leaving the hospital committed suicide.																				
58	M	12	0.5	1-16-23	105.8	.....	?	?	?	?	?	0	?	.....	5.3	.....	.....	+++	.....	
				1-23-23	107.7	.....	35	38	83	1039	65	34	31	0	.....	.....	0	.....	0	.....
				5-12-23	112.0	6.2	77	65	182	2206	133	38	95	0	.....	30	.....	0	.....	

CHART No. 1—Continued

No.	Sex	Age	Duration Yrs.	Date	Net Wt., lbs.	Gain in Wt.	DIET					Insulin "G."	Tol- erance "G."	Ile- tin Units	Urine Sugar		Blood Sugar %	Acidosis	
							C.	P.	F.	Cal.	"G."				%	Grams		Acetone	NH3
59	M	55	3	1-16-23	114.3	.....	?	?	?	?	?	0	?	.....	2.6	.....	.....	++	.....
				1-24-23	116.8	.....	55	43	118	1454	92	48	.....	0	.....	.....	0	.....	
				7-15-23	128.7	14.4	77	65	182	2206	133	20	.....	0	.....	.....	0	.....	
61	M	61	4	1-20-23	115.0	.....	?	?	?	?	?	0	?	.....	5.1	130	.....	++	2.0 Gm.
				2-20-23	121.5	6.5	77	65	182	2206	133	119	14	.....	0	.....	.....	0	.....
				3-29-23	130.0	15.0	74	83	116	1690	134	0	?	.....	.5	.....	.....	?	.....
When he returned to New York this patient discontinued taking insulin because of the cost.																			
62	F	53	16	1-21-23	93.8	.....	?	?	?	?	?	0	?	.....	.3	.....	.....	+	.....
				1-24-23	91.7	.....	35	38	83	1039	65	39	26	.....	0	.....	.....	0	.....
				7-15-23	102.4	8.4	83	56	187	2237	134	117	17	.....	0	.....	.....	0	.....
63	M	40	1	1-22-23	149.8	.....	?	?	?	?	?	0	?	.....	5.5	.....	.....	++	.....
				1-29-23	152.0	.....	77	45	169	2009	120	43	77	.....	0	.....	.17	0	.....
				2-18-23	147.0	.....	77	65	182	2206	133	19	114	.....	0	.....	.13	0	.....
64	F	70	10	1-29-23	80.0	.....	?	?	?	?	?	0	?	.....	.8	.....	.....	++	.....
				2-7-23	81.0	.....	77	45	169	2009	120	67	53	.....	0	.....	.....	0	.....
				6-9-23	110.0	30.0	77	65	182	2206	133	43	90	50	0	.....	.....	0	.....
65	M	60	5	1-31-23	127.4	.....	?	?	?	?	?	0	?	.....	3.2	.....	.....	++	.....
				1-4-23	128.0	.....	77	65	182	2206	133	34	99	.....	0	.....	.....	0	.....
				6-12-23	157.0	29.6	87	68	204	2507	147	17	130	20	0	.....	.....	0	.....
66	F	10	1	2-2-23	73.0	.....	?	?	?	?	?	0	?	.....	4.8	.....	.....	coma	.....
				2-13-23	73.0	.....	65	62	131	1687	114	49	65	.....	0	.....	.....	0	.....
				6-15-23	90.0	17.0	77	65	182	2206	133	40	93	32	0	.....	.....	0	.....
This patient was doing very well until there was a sudden decrease in the value of the ileitin unit. She passed quantities of sugar in the urine, became discouraged, and was again in severe acidosis upon her second admission June 23, 1923.																			
				6-23-23	.....	.....	?	?	?	?	?	0	?	.....	5.1	.....	.....	+++	.....
				7-15-23	83.1	.....	77	65	182	2206	133	36	97	40	0	.....	.....	0	.....

CHART No. 1—Continued

No.	Sex	Age	Duration Yrs.	Date	Net Wt. lbs.	Gain in Wt.	Diet				Insul- in "G."	Toler- ance "G."	Ile- tin Units	Urine Sugar		Blood Sugar %	Acidosis			
							C.	P.	F.	Cal.				"G."	%		Grams	Acetone	NH3	
67	M	67	10	2-3-23	141.0	-----	?	?	?	?	?	0	?	-----	.6	-----	-----	†	-----	
				2-20-23	143.5	-----	77	65	182	2206	133	40	93	0	-----	-----	0	-----	0	-----
				3-1-23	142.3	1.3	77	65	182	2206	133	40	93	0	-----	-----	0	-----	0	-----
68	F	25	3.5	2-5-23	73.6	-----	?	?	?	1000	73	0	73	-----	0	-----	0	-----		
				6-7-23	109.0	35.4	77	65	182	2206	133	49	84	0	-----	0.09	0	-----	0	-----
69	M	48	11	2-7-23	107.3	-----	?	?	?	?	?	0	?	-----	3.4	71.4	-----	0	-----	
				2-11-23	101.3	-----	77	65	182	2206	133	40	93	0	-----	-----	0	-----	0	-----
This patient was a drug addict. He died Feb. 19, 1923, following an acute respiratory infection and probable over-dosage of morphine.																				
70	F	50	10	2-8-23	138.9	-----	?	?	?	?	?	0	?	-----	.9	-----	-----	††	-----	
				2-13-23	139.4	-----	25	31	74	890	50	27	23	0	-----	-----	0	-----	0	-----
				6-8-23	146.0	7.1	57	44	178	2006	120	35	85	0	-----	-----	0	-----	0	-----
This patient had a steadily growing colloid goitre which was large enough to produce marked pressure on the trachea and oesophagus. It was removed April 11, 1923, under ether anaesthesia, without complications.																				
71	M	58	?	2-9-23	-----	-----	?	?	?	?	?	0	?	-----	†††	-----	-----	coma	-----	
				This patient had had diabetes for a long time. It was complicated by an injured leg, and suppuration had set in. He was seen in another hospital after being in deep coma all night. One dose of insulin was given. He died 15 minutes later.																
72	M	62	1.5	2-9-23	143.3	-----	?	?	?	?	?	0	?	-----	4.6	-----	0	-----		
				2-15-23	148.0	-----	35	38	83	1039	65	65	0	0	-----	-----	0	-----	0	-----
				6-16-23	158.0	14.7	77	65	182	2206	133	34	99	0	-----	-----	0	-----	0	-----
73	M	28	2	2-12-23	124.0	-----	?	?	?	?	?	0	?	-----	.5	-----	0	-----		
				2-21-23	131.0	-----	77	65	182	2206	133	40	93	0	-----	-----	0	-----	0	-----
A tonsillectomy was performed Feb. 22, 1923.																				
							100	80	240	2880	170	0	170	-----	0	-----	-----	0	-----	



CHART No. 1—Continued

No.	Sex	Age	Dur- ation Yrs.	Date	Net Wt. lbs.	Gain in Wt.	Diet				Insul- in "G"	Toler- ance "G"	Ile- tin Units	Urine Sugar		Blood Sugar %	Acidosis	
							C.	P.	F.	Cal.	"G"			%	Grams		Acetone	NH <sub>3</sub>
74	F	69	6	2-15-23 2-22-23 6-13-23	122.2 122.5 131.5	9.3	? 35 77	? 38 45	? 83 169	? 1039 2009	? 65 120	? 27 86	..... ..... 40	3.5 0 0	..... ..... .....	..... ..... .....	†† 0 0	.....
75	M	19	0.5	2-16-23	118.5	.....	? This patient was mentally deficient. He left the hospital after one day without our consent.	? .....	? .....	? .....	? .....	? .....	.....	††† .....	.....	.....	† .....	.....
76	F	72	11	2-17-23 2-27-23 3-23-23	99.3 100.3 102.7	..... 3.4	? 75 65	? 63 62	? 173 131	? 2109 1687	? 129 114	? 72 72	..... ..... .....	5.7 0 0	..... ..... .....	..... 0 0.16	0 0 0	.....
77	F	57	5	2-19-23 3-5-23	168.5 168.2	.....	? 77	? 43	? 165	? 1965	? 118	? 93	..... .....	0 0	..... .....	..... .....	0 0	.....
78	M	53	6	2-21-23 3-6-23 7-1-23	95.5 98.0 109.8	..... 14.3	? 77 77	? 65 65	? 182 182	? 2206 2206	? 133 133	? 57 85	..... ..... .....	1.7 0 0	..... ..... .....	..... ..... .....	0 0 0	.....
79	M	66	1	2-24-23 3-5-23 6-11-23	124.5 123.6 126.5	..... 2.0	? 77 77	? 65 65	? 182 182	? 2206 2206	? 133 133	? 87 110	..... ..... 28	0 0 0	..... ..... .....	..... ..... .....	0 0 0	.....
80	M	58	0.5	2-27-23 3-6-23	138.3 140.9	2.6	? 53	? 43	? 118	? 1446	? 88	? 88	..... .....	0 0	..... .....	..... .....	0 0	.....
81	F	62	12	3-7-23 3-12-23 6-12-23	106.5 107.7 117.0	..... 10.5	? 77 77	? 65 65	? 182 182	? 2206 2206	? 133 133	? 63 101	..... ..... 38	2.5 0 0	..... ..... .....	..... ..... .....	† 0 0	.....
82	M	45	1.5	3-7-23 3-28-23 6-6-23	116.5 126.6 137.5	..... 21.0	? 77 100	? 65 96	? 182 250	? 2206 3034	? 133 181	? 63 135	..... ..... 54	1.9 0 0	..... ..... .....	..... ..... .....	††† 0 0	.....
83	F	37	1.5	3-8-23 6-11-23	129.2 137.5	8.3	55 67	43 64	118 165	1454 2009	92 120	92 101	..... 22	0 0	..... .....	..... .....	0 0	.....

This patient consented to take insulin for two days only and then left the hospital.

CHART No. 1—Continued

No.	Sex	Age	Dur- ation Yrs.	Date	Net Wt. lbs.	Gain in Wt.	Diet				Insul- in "G."	Toler- ance "G."	Ile- tin Units	Urine Sugar		Blood Sugar %	Acidosis		
							C.	P.	F.	Cal.				"G."	%		Grams	Acetone	NH3
84	M	43	5	3-8-23	108.5	.....	?	?	?	?	?	?	.....	2.2	.....	.....	++	.....	
				3-11-23	108.5	.....	77	65	182	2206	133	0	.....	0	.....	.....	0	.....	
				5-27-23	119.0	10.5	87	78	253	2937	157	43	.....	0	.....	.....	0	.....	
85	F	51	7	3-9-23	127.0	.....	?	?	?	?	?	?	.....	7.0	.....	.....	0	.....	
				3-22-23	131.0	4.0	67	63	180	2140	122	60	48	0	.....	.....	0	.....	
This patient left the hospital without our consent after taking insulin for 19 days.																			
86	M	33	8	3-10-23	99.0	.....	?	?	?	?	?	?	.....	+++	.....	.....	++	.....	
				3-17-23	105.0	.....	55	43	118	1454	92	25	.....	0	.....	.....	0	.....	
				6-11-23	127.0	28.0	100	98	248	3024	182	145	44	0	.....	.....	0	.....	
87	F	63	4	3-12-23	170.0	.....	?	?	?	?	?	?	.....	3.0	13.0	.....	+++	.....	
				3-19-23	172.5	.....	77	65	182	2206	133	50	40	0	.....	.....	0	.....	
				7-15-23	152.0	.....	71	63	180	2156	126	109	17	0	.....	.....	0	.....	
This patient was readmitted to the hospital on June 9, 1923, following gross neglect of her diet and discontinuing insulin. She had a severe attack of sciatica, and is now improving, following a tonsillectomy on July 9, 1923.																			
88	M	33	0.5	3-14-23	115.6	.....	?	?	?	?	?	?	.....	1.1	.....	.....	+	.....	
				4-1-23	117.2	.....	106	98	246	3030	188	30	158	.....	0	.....	.....	0	.....
				6-8-23	132.0	16.4	106	98	246	3030	188	20	168	.....	0	.....	.....	0	.....
89	M	54	7	3-15-23	157.5	.....	?	?	?	?	?	?	.....	.9	.....	.....	0	.....	
				3-19-23	154.5	.....	77	65	182	2206	133	87	46	.....	0	.....	.....	0	.....
				5-7-23	167.0	9.5	104	99	246	3026	186	22	164	.....	0	.....	.....	0	.....
90	M	49	5	3-20-23	147.7	.....	?	?	?	?	?	?	21	4.0	7.0	.....	+++	1.8 Gm.	
				3-26-23	147.7	.....	77	65	182	2206	133	85	68	.....	.08	0	.....	0	.....
				4-29-23	170.0	22.3	84	78	233	2745	152	90	62	.....	0	.....	.....	0	.....
91	M	23	3	3-21-23	108.0	.....	?	?	?	?	?	?	.....	+++	.....	.....	++	.....	
				3-28-23	118.0	.....	55	43	118	1454	92	86	6	.....	0	.....	.....	0	.....
				7-15-23	132.5	24.5	97	82	314	3542	176	81	95	.....	0	.....	.....	0	.....

CHART No. 1—Continued

No.	Sex	Age	Duration Yrs.	Date	Net Wt. lbs.	Gain in Wt.	Diet				Insul- in "G."	Toler- ance "G."	Insu- lin Units	Urine Sugar		Blood Sugar %	Acidosis	
							C.	P.	F.	Cal.	"G."			%	Grams		Acetone	NH <sub>3</sub>
92	M	57	11	3-22-23 3-26-23 6-18-23	135.5 138.5 164.0	28.5	?	?	?	?	?	0	?	†	.....	.....	0	.....
							104	96	253	3077	185	41	144	0	.....	.14	0	.....
							100	100	200	2600	178	34	144	0	.....	.....	0	.....
93	M	54	3	3-24-23 4-1-23	211.0 207.5	.....	?	?	?	?	?	0	?	4.2	.....	.....	†††	.....
							77	65	182	2206	133	20	113	0	.....	.....	0	.....
							Because of his marked overweight we advised this patient to take a 1500 calorie diet without insulin.											
94	M	42	1	3-6-23	166.3	.....	77	65	182	2206	133	0	133	0	.....	.15	0	.....
							Insulin was given for 9 days to reduce the blood sugar.											
							77	65	182	2206	133	0	133	0	.....	.13	0	.....
95	F	53	1	4-3-23 4-20-23	163.5 162.6	.....	77	45	169	2009	120	0	120	0	.....	.16	0	.....
							77	45	169	2009	120	19	101	22	.....	.12	0	.....
							Insulin was given for 7 days only to reduce the blood sugar.											
							77	45	169	2009	120	0	120	0	.....	.16	0	.....
96	F	60	2.5	3-20-23 4-4-23 6-18-23	155.0 150.5 157.0	.....	?	?	?	?	?	0	?	7.4	.....	.....	†	.....
							82	57	190	2266	134	20	114	0	.....	.....	0	.....
							99	96	250	3030	180	24	156	28	.....	.....	0	.....
97	F	58	12	4-7-23 4-14-23	97.4 98.6	.....	?	?	?	?	?	0	?	4.9	.....	.....	0	.....
							55	43	118	1454	92	40	52	0	.....	.....	0	.....
98	M	44	4	4-8-23 4-14-23 6-6-23	97.6 111.6 127.0	29.4	?	?	?	?	?	0	?	4.6	.....	.....	†††	.....
							77	65	182	2206	133	81	52	0	.....	.....	0	.....
							77	65	182	2206	133	81	52	95	.....	.....	0	.....
99	M	56	5	4-13-23 4-17-23 7-1-23	153.0 153.4 155.0	.....	?	?	?	?	?	0	?	5.7	.....	.....	0	.....
							77	65	182	2206	133	50	83	0	.....	.....	0	.....
							91	79	257	2993	162	37	125	0	.....	.....	0	.....
100	M	31	4	4-17-23 6-6-23	125.0 141.0	16.0	?	?	?	?	?	13	128	0	.....	.....	0	.....
							96	104	250	3050	181	34	147	0	.....	.....	0	.....

## OLD AND NEW TREATMENT OF DIABETES FROM A STATISTICAL POINT OF VIEW.

By K. A. HEIBERG, Copenhagen.

Petrén's results from his attempts at modifying the diet in treatment of diabetes in the way of a low amount of protein and a high content of fat have now been published in detail.<sup>1</sup>

Evidently, this modification of the so-called "old system"—for Petrén's diabetes diet may obviously be counted with this form of diabetic diet—does not record better results than the original "old system."

Although a statistical comparison of the various methods of treatment generally is somewhat unreliable, it is possible, nevertheless, to compare the results which Petrén attains with the experiences of Allen and Sherrill.<sup>2</sup>

As the course of diabetes in young persons without insulin treatment is so rapid, only relatively small groups are necessary in order to show a difference in mortality, as in the following table.

The table shows the results, as regards *mortality during the first year of disease*, from Petrén's material and from Allen and Sherrill's series of investigations, including only cases occurring in the 11th to the 30th year of age, and *treatment commencing during the first year of disease*. The two series contain cases of equal gravity, and for Petrén's material no details have been given beyond that it is practically the entire material, i. e., every case that could be classed as true diabetes within the limits of the said two decennia of life. From Petrén's material a relatively greater number of grave, but practically untreated cases, have been excluded than from that of Allen and Sherrill, so Petrén has not been prejudiced in this respect. Besides, in conformity with Petrén, I have included among the ones who were treated, but *not* among the ones who died, during the first year of disease two cases of death during the first year, which Petrén does not acknowledge as caused by diabetes, furthermore, one patient about whose progress information is missing.

Mortality during 1st year of disease in cases of diabetes during 2nd and 3rd decennium (11th to 30th years of age), treatment commencing during first year of disease.

	Number of cases	Died during 1st year	Mortality percen- tage of 1st year of observation
Treatment commenced during 1st year of disease in Petrén's material .....	33	18	54
Treatment commenced during 1st year of disease in Allen and Sherrill's series of investigations.....	43	4	9

We can draw no other conclusion than that the results of Allen and Sherrill are better.

#### REFERENCES.

1. Petrén, K. *Diabetes-Studier*. Copenhagen, 1923.
2. Allen, F. M., and Sherrill, J. W. *J. Metabolic Research*, 1, 1922, 377-456. Clinical observations on treatment and progress in diabetes.



## STUDIES IN INORGANIC METABOLISM.

### III. THE SIGNIFICANCE OF PHOSPHATES IN THE PRODUCTION OF TETANY.

BY FRANK P. UNDERHILL, ERWIN G. GROSS AND  
WILLIAM COHEN.\*

*From the Department of Pharmacology and Toxicology, Yale University,  
New Haven, Conn.*

Symptoms of tetany have been observed clinically in a number of widely differing conditions, such as:

1. Disease or absence of the parathyroid glands.
2. In infants as a clinical entity, often associated with rickets.
3. Following or complicating infectious diseases.
4. Tetany in the course of pregnancy or lactation.
5. After prolonged hyperpnea.
6. Overdosage with sodium bicarbonate.

Experimentally, too, tetany resembling idiopathic tetany in man has been produced in numerous ways, and in recent years, there has been considerable research into the physiologic phenomena underlying the increased irritability of nervous and muscular tissue in these conditions. These researches have resulted in the formation of essentially three independent, but not necessarily contradictory theories concerning the metabolic disturbances in tetany.

The first of these holds that the essential change is a disturbance of the salt metabolism, particularly that of calcium. Jacques Loeb,<sup>1</sup> in a paper on the production of muscular twitching by electrolytes, found that the injection into the animal body of any salt likely to precipitate calcium, produces twitching of the muscles, and suggested that "abnormal conditions might arise in which an increase of such acids in the circulation could diminish the circulation of calcium in the body. The necessary outcome would be muscular twitching. In that case, the administration of calcium salts might cure the disease." He showed experimentally that the immersion of a muscle nerve preparation in a solution of a salt which precipitates or renders inactive the calcium gives rise to twitchings which are suppressed by the addition of fresh calcium to the solution, or transfer to a calcium solution.

\* A portion of the data are taken from the thesis of William Cohen, M.D., presented in partial fulfillment of the requirements for the degree of M.D., Yale University, 1923.

A further great stimulus to the study of the relationship of calcium to tetany was given by the observation of Sabbatini,<sup>2</sup> that a solution of calcium applied to the brain diminishes its irritability to electrical stimulation, but substances that caused a precipitation of calcium, such as oxalates or citrates, brought about an increased irritability.

Quest,<sup>3</sup> Cohn,<sup>4</sup> and Aschenheim,<sup>5</sup> stimulated by the work of Sabbatini, examined the brains of children dying from tetany and from other causes. Although they found a considerable diminution of calcium in some of the tetany cases, there was little regularity of the results, so that no definite conclusions could be drawn. Quest also tried to produce electrical hyperexcitability in animals by giving them a calcium-free diet and reported that the excitability of calcium starved dogs is remarkably increased.

Among the first attempts to study quantitatively the calcium of the blood in conditions of tetany were those made by Neurath,<sup>6</sup> Cattaneo,<sup>7</sup> and Longo.<sup>8</sup> Neurath used Wright's method, which depends upon the amount of oxalate that must be added to prevent the coagulation of blood. This method gives relative but not absolute figures. In his examination of the blood of fifteen infants with various manifestations of tetany of more or less severity, he found in general a reduction of calcium. Cattaneo and Longo made gravimetric determinations of blood calcium of a few normal children and a few with tetany. Both found great reductions of calcium in tetany, although their figures vary tremendously.

Another great step forward was taken when MacCallum and Voegtlin<sup>9</sup> reported that in the tetany of parathyroidectomized dogs, there is a marked reduction of calcium, and that the symptoms could be promptly, though temporarily, relieved by the intravenous administration of calcium salts. A few years later, MacCallum and Vogel<sup>10</sup> reported similar observations, and also stated that although the administration of parathyroid extract does not increase the calcium content of the blood, if extirpation of the parathyroids has been incomplete, so that tetany does not appear, the calcium content of the blood is normal.

In 1916, Marriott and Howland<sup>11</sup> reported that in nephritis with acidosis there is a great accumulation of inorganic phosphates in the blood serum, and at the same time, a reduction of calcium. On the basis of these findings, Binger<sup>12</sup> was able to produce tetany in dogs by the intravenous administration of phosphate solutions and reported a considerable reduction of blood calcium.

In 1917, Howland and Marriott<sup>13</sup> made some accurate studies of the serum calcium in a number of cases of normal infants and infantile tetany. They found that in all cases of tetany the calcium was reduced to 7.0 mg. or less, the normal value being 10.0 mg. per 100 cc. serum. Since then these findings have been confirmed by many other investigators. Hastings and Murray,<sup>14</sup> following the blood calcium at various stages after parathyroidectomy, showed that the concentration of calcium commences to decrease rapidly soon after removal of these glands, and in a few days reaches a value which is about 40 per cent. of the normal. When the serum calcium reaches a concentration of approximately 7.0 mg. (11 mg. per 100 cc. being the normal), tetany supervenes. These findings are consistent with the values in infantile tetany and two parathyroidectomized

dogs as found by Howland and Marriott,<sup>15</sup> and in a case of adult tetany recently reported by Barach and Murray.<sup>16</sup>

In general, then, a diminution of blood calcium has been regarded by most observers as a constant and fundamental disturbance in idiopathic tetany and that caused by parathyroidectomy.

The second theory regarding the etiology of tetany emphasizes a disturbance in the acid base equilibrium. MacCallum and Voegtlin,<sup>9</sup> in studying the metabolism of parathyroidectomized dogs, reported among other things, that there was an increased amount of ammonia in the blood; also an increased output of total nitrogen in the urine with a relative increased ammonia ratio. They concluded that "much of this affords evidence of the existence of some type of acid intoxication." They therefore administered sodium bicarbonate in an attempt to relieve the symptoms of tetany in parathyroidectomized dogs, but found to their surprise, that alkalies exaggerated the symptoms. Similar observations as to the increased ratio of ammonia nitrogen in the urine of parathyroidectomized dogs were reported by Underhill and Saiki<sup>17</sup> and Cooke.<sup>18</sup> Morel,<sup>19</sup> too, in 1911, stated that a condition of acidosis prevailed following parathyroidectomy, basing his opinion chiefly on the high concentration of ammonia and lactic acid in the blood.

Recently the pendulum has swung to the opposite direction. In 1915, Wilson, Stearns, and Janney<sup>20</sup> again called attention to the metabolic changes noted above, but in offering an explanation for these phenomena, suggested that there might be a tendency toward alkalosis in parathyroidectomy, as further shown by the fact that alkalies have been known to increase the symptoms of tetany. In this preliminary report, they offered the further corroborative evidence that in their hands, the introduction of acids afforded prolonged periods of relief in animals showing tetany after parathyroidectomy. Shortly afterwards the same authors<sup>21</sup> presented more detailed evidence which tended to show that after parathyroidectomy, a "condition of alkalosis may develop, which is neutralized by the acid products formed by the muscular activity incident to tetany. Periodic variation in the 'non-volatile' acid base equilibrium seems to accompany the periodic attacks." They based their conclusions on a study of the dissociation constant of oxyhemoglobin, the CO<sub>2</sub> content of the alveolar air, the hydrogen ion concentration of the blood, the ammonia content of the blood, and the total acidity and hydrogen ion concentration of the urine. Injection of acids and also calcium salts relieved the tetany. They suggested that part of the beneficial action of calcium salts might be due to a relative increase in acid radicals caused by their administration.

The alkalosis theory was greatly strengthened in 1918, when McCann<sup>22</sup> reported that not only after parathyroidectomy, but also following operations on the stomach which exclude the acid secreted from the duodenum, there is a marked increase in the CO<sub>2</sub> combining power of the plasma, coincident with the development of tetany. He also found that intravenous injection of acid and the introduction of acid through the duodenum relieved the tetany and therefore concluded that tetany is a condition of alkalosis in which a disproportion between the rates of secretion of acids and alkalies by the gastro intestinal tract might be a factor. MacCallum

and his co-workers<sup>22</sup> obtained practically similar results. They found that when the pylorus of the dog is obstructed and the gastric juice with its HCl is constantly removed, there ensues a decrease in the chlorine of the plasma, and as a result of this, there is an increase in the alkali reserve which may become extreme. This produces exaggerated electrical excitability of the nerves, there are spontaneous twitchings, and in most cases, violent convulsions which lead to death. This can be prevented by the introduction into the duodenum of NaCl or the symptoms made to disappear by the intravenous administration of NaCl. Calcium determinations by a dialyzing method, showed no change in the calcium content of the plasma. They suggested that possibly the disturbed equilibrium of acids and bases in itself is the cause of symptoms. Clinical evidence pointing towards a condition of alkalosis in gastric tetany has also been brought forward by Grant.<sup>24</sup> The latter reported six cases of tetany in adults, in all of which there was an acid base disturbance. Of these, three cases were due to obstruction of the pylorus, and in two of these, the plasma CO<sub>2</sub> was greatly increased. Calcium determinations in one of the cases showed normal values.

Another type of tetany which has been attributed to an alkalosis is that caused by hyperpnea. Collip and Backus<sup>25</sup> observed tetany in a few of fifteen individuals following periods of voluntary forced breathing. Grant and Goldman<sup>26</sup> also were able, by voluntary forced respiration, to produce symptoms of tetany as manifested by carpopedal spasm, Chvostek's sign, Trousseau's sign, Erb's sign, and in one instance tetanic convulsions. Accompanying this condition, they found a fall in the alveolar CO<sub>2</sub> tension, a reduction in the hydrogen ion concentration of the blood, a reduction of plasma CO<sub>2</sub>, a change in the reaction of the urine to the alkaline side, and a decreased excretion of ammonia. The calcium content of the serum was slightly increased. They explain the whole picture on the basis that the carbonic acid in the blood is decreased by washing out of CO<sub>2</sub> faster than the sodium bicarbonate is decreased by excretion or neutralization. There is thus a relative excess of the

alkali and an increase in the ratio  $\frac{\text{NaHCO}_3}{\text{H}_2\text{CO}_3}$ . Grant<sup>24</sup> reported a case of tetany due to hyperpnea in a female patient with hysteria.

Finally, another group of cases of tetany supposedly due to alkalosis are those following the therapeutic administration of sodium bicarbonate. Tileston<sup>27</sup> reported severe tetany in a case of Weil's disease, following an intravenous injection of sodium bicarbonate, producing a plasma CO<sub>2</sub> of 80 volumes per cent. Howland and Marriott<sup>28</sup> reported three cases of tetany in children, brought on by the intravenous injection of NaHCO<sub>3</sub> to relieve acidosis in diarrhea. All of these were associated with a marked reduction of the serum calcium. This is the only instance, so far as we know, of a reduction in calcium following sodium bicarbonate administration. Harrop<sup>28</sup> recently reported a case of typical tetany in an adult with nephritis who had been given a total of 60 gms. of sodium bicarbonate intravenously in divided doses on two consecutive days. His condition was associated with an increased CO<sub>2</sub> combining power of the plasma,



a diminished alveolar  $\text{CO}_2$  tension, but without a decrease in the calcium content of the plasma.

The alkalosis theory, while very interesting and exceedingly popular, has, however, met with considerable opposition. MacCallum,<sup>23</sup> Togawa,<sup>24</sup> Hastings, and Murray,<sup>14</sup> and Underhill and Nellans<sup>25</sup> were unable to find any change in the reaction of the blood, or increase in the  $\text{CO}_2$  combining power of the plasma in tetany following parathyroidectomy. Also in tetany following pyloric obstruction, Hastings and his co-workers<sup>21</sup> could find no change in the reaction of the blood. Greenwald<sup>22</sup> especially has most vigorously opposed the theory that an alkalosis is the cause of tetany in any of the conditions mentioned thus far. He emphatically states that no change in the pH of the blood has ever been accurately observed following the administration of sodium bicarbonate or after hyperpnea. He ascribes the untoward effects in the former condition as being due to "sodium poisoning," due either to an excess of sodium, or a disturbance of the normal equilibrium between sodium and other ions. As for tetany, following hyperpnea, this is not due to an alkalosis, *per se*, but to a tissue anoxemia, resulting from increased stability of the oxyhemoglobin. Greenwald's views are rapidly gaining many adherents.

The third view regarding the etiology of tetany is the so-called "toxic" theory, brought forward by Paton and Findlay,<sup>26</sup> who claim that following parathyroidectomy, a highly toxic substance of protein origin is found in the blood and urine. More specifically, they have found an increase in guanidin and methyl guanidin and suggest that these may be the offending agents. They also corroborated the observation of other investigators that the administration of these substances results in hyperexcitability very similar to that seen in tetany. Considerable weight has been added to the toxic theory by the work of Luckhardt and Rosenbloom<sup>27</sup> who reported that by continuous intravenous injection of calcium-free Ringer's solution, they were able to keep tetany dogs alive for fifty-one days. This would seem to indicate that the diuresis thus produced washed out some toxic substance or substances. While extraordinarily interesting, sufficient work along this line has not as yet been done to establish its validity.

Recently a very interesting theory has been presented by Freudentberg and Gyorgy<sup>28</sup> in an attempt to correlate all forms of tetany as being due to a calcium deficiency in the tissues, with a resulting hyperexcitability. They based their theory partly on the observations that calcium union with colloids may be influenced by changes in hydrogen ion concentration, by changes in calcium ion concentration, by such substances as phosphates and bicarbonate ions, and by protein derivatives with free amido groups as guanidin, creatin, etc. In conditions in which there is an increase in the ratio of  $\text{NaHCO}_3$  to  $\text{H}_2\text{CO}_3$ , free calcium ions in the blood decrease, being added to the total of calcium bound to colloids in the blood. This resulting disturbance of the equilibrium between ionized blood calcium and calcium bound with tissue colloids, causes calcium to enter the blood stream from the tissues, thus depleting the latter and producing increased irritability. The calcium content of the blood should therefore be increased, and, as a matter of fact, very slight increases in serum calcium



have been reported in the tetany due to hyperpnea<sup>26</sup> and after pyloric obstruction.<sup>21</sup> The occurrence of tetany without an acid base disturbance is explained by the action of protein derivative substances with free amido groups. These restrict calcium union with colloids, thus decreasing tissue calcium and causing increased irritability. Calcium enters the blood from the tissues, causing presumably an increased blood calcium content at the outset. However, by calcium excretion, the equilibrium between blood and tissues is reestablished, the blood calcium being adjusted to the low level of tissue calcium. In this process, the per cent. of diffusible calcium in the blood should not be altered and it is interesting to note that Meysenburg and McCann<sup>20</sup> have found the same per cent. of diffusible calcium in normal blood and in the calcium-poor blood of parathyroidectomized dogs.

This theory, too, while it is very interesting and ingenious, is based almost exclusively on a theoretical foundation and has also met with specific objections.

Many investigations have been conducted recently in an endeavor to determine the relation of some of the different organic constituents in the serum, besides calcium, to the production of tetany. Among these, the phosphates particularly have been regarded as possessing special significance. Greenwald<sup>27</sup> discovered a phosphate retention in tetany following parathyroidectomy. Jepson and Klercker<sup>28</sup> found that by feeding 0.20 gm. of phosphorus pentoxide per kilogram body weight to normal infants and 0.10 gm. to latent spasmophilic infants, symptoms similar to those of active spasmophilia were produced. These results were often evident in a few hours when potassium salts were employed; while larger quantities of sodium salts were needed to produce the same effects. Kramer, Tisdall, and Howland<sup>29</sup> have recently reported data on eight children with infantile tetany, in some of whom the concentration of inorganic phosphorus of the serum was slightly above normal. A very interesting investigation in this connection was that of Binger,<sup>12</sup> referred to above; namely, the production of tetany in dogs by the intravenous injection of ortho-phosphates. His conclusions were as follows:

"When the phosphate solution is injected in amounts equivalent to 150 mgs. phosphorus per kilogram, the serum calcium drops from its normal value to 10 mgs. per 100 cc. to approximately 6 mgs. At this level, a condition of tetany supervenes, provided that the neutral or alkaline salts have been injected. With acid phosphate solutions, the calcium drop occurs unaccompanied by tetany. Toxicity is due to a specific action of the phosphate ions in combination with the reaction of the solution in which they exist."

These results were of great interest inasmuch as two of the conditions said to obtain in idiopathic tetany were reported; namely, (1) a reduction of serum calcium and presumably, (2) a disturbance of the acid base equilibrium, tending to produce an alkalosis. In view of these facts, it seemed desirable and important, therefore, to carry on further investigations of the same kind, employing other salts in addition to sodium phosphate, with the aim of evaluating the relative importance of the agents used and the changes observed.

### *Experimental Procedure.*

Rabbits and dogs were used as the experimental animals. The phosphate solutions employed with rabbits were prepared by titrating  $H_3PO_4$  with NaOH, and  $H_3PO_4$  with KOH; making them up to the desired hydrogen ion concentration by using Clark and Lub's<sup>40</sup> standards. Sodium phosphates were used in the strength of normal solutions, while half normal potassium phosphate solutions were employed on account of the marked depressant action of the potassium ion. The phosphorus content of all solutions was determined by the uranium acetate method.<sup>41</sup> Calcium determinations were made on whole blood by an adaptation from Kramer and Tisdall's method.<sup>42</sup> Injections were made into the external ear vein. Blood for calcium determinations was obtained from the opposite ear by puncturing the vein and collecting the blood as it dripped into a known weight of water.

The solutions employed with dogs were the primary and secondary sodium and potassium phosphates, which were injected in isotonic solutions unless otherwise noted.

The sodium solutions were injected intravenously as rapidly as possible, usually requiring from 10-15 minutes for completion. The potassium salts were injected intraperitoneally due to their depressant action on the heart. Blood samples were aspirated from the external jugular. The chlorides were determined according to the method of Whitehorn,<sup>43</sup> inorganic phosphates according to the method of Briggs,<sup>44</sup> and calcium, potassium and sodium according to the method of Kramer and Tisdall.<sup>45</sup> The magnesium was determined by an adaptation of the Bell-Doisy phosphate method. Hemoglobin was determined according to the method of Cohen and Smith, and sodium and potassium in the urine by the method described in Hawk.<sup>46</sup>

### *Experiments with Rabbits.*

#### *Is Tetany Produced by Injections of Phosphates?*

Experiments were first conducted to determine whether or not tetany could be produced and also the significance of the hydrogen ion concentration of the solutions employed. In general, the results obtained coincided almost exactly with those of Binger's. Solutions with a pH of 7.4 or above invariably produced tetany while those with a pH of 6.4 or less did not, even though much larger doses of the latter were employed. In one instance, however, a solution of sodium phosphate with a pH of 6.4 did excite typical tetany.

In these experiments, shivering and occasional muscular contractions were not regarded as conclusive evidence of tetany, since these phenomena occur in a normal rabbit when it is merely tied down. The criteria for tetany as adopted in these investigations were as follows: exaltation of reflexes; generalized fine and

coarse tremors, followed by clonic contractions and eventually convulsive fits, and occasional tonic convulsions in which the animal lay in a position of opisthotonus; also alternation of periods of considerable violence with intervals of marked depression, and prostration; and finally, extreme drooling salivation. The pictures obtained were so striking and clean cut, that it seemed unnecessary to employ any methods for studying electrical reactions for evidences of heightened neuro-muscular irritability.

A few protocols of some of the experiments are as follows:

*Experiment 6.* Sept. 1. Weight of rabbit, 2060 gms.

Solution N NaOH.H<sub>3</sub>PO<sub>4</sub>; pH 7.4.

Uranium acetate titration = 4.47 mg. P. per cc.

11:25-11:55—Injected 53 cc.

11:25—Pulse 140, respiration 60.

11:30—Animal begins to shiver.

11:35—Fine fibrillary twitching of forelimbs; beginning to salivate.

11:40—Generalized fine tremors, salivation more marked, forelimbs rigidly extended.

11:55—Generalized convulsive fit. Animal was released from the board and placed on the floor. Attempts to rise, but falls back. Limbs appear to be very weak. Remains lying on its side with head thrown rigidly back, limbs extended, and showing coarse tremors and generalized muscular twitching; respiration slower (40) deep, and labored; salivation very profuse.

12:00—Convulsions have ceased; animal appears relaxed, respirations easier and more rapid (130).

12:03—Animal prodded and is able to rise on all fours and hop a few steps.

12:10—Suddenly had another convulsion; lies on its side in position of opisthotonus with generalized coarse tremors and extension of limbs; respirations 180; salivation again very profuse. Convulsion lasted 3 minutes.

12:20—Animal appears extremely prostrated; generalized fine tremor, most marked in facial muscles; respirations 100. Animal not observed again until

3:00—Found sitting up, apparently quite well. Upon picking up the rabbit and placing it on a table, it immediately became very stiff and tense, and then fell over on its side and had another convulsive fit, with profuse salivation, and accompanied by a peculiar whining cry. Respiration 200. Attack lasted about 2 minutes and then ceased except for tremors of forelimbs.

- 3:20—Animal again shows generalized fine tremors with drooling salivation.
- 3:30—Excitability greatly exalted; any external stimulus, such as slight prodding, barking of a dog, or other loud noise causes marked excitement and at times throws animal into convulsive fit again.
- 3:40—Animal returned to its cage.  
Following morning animal was found dead in a position of opisthotonus.  
Total mg. P injected = 236.9.  
mg. P per kilo injected = 115.

*Experiment 8.* Sept. 9. Weight of rabbit, 2200 gms.

Solution N NaOH.H<sub>3</sub>PO<sub>4</sub>, pH 8.8.

Uranium acid titration = 4.25 mg. P per cc.

- 10:15-11:00—Injected 55 cc.
- 10:25—Animal begins to shiver and show fine tremors in forelimbs.
- 10:30-11:00—Occasional generalized clonic convulsions and coarse body tremors with extension of all four limbs. Slight salivation. Respirations deep and labored.
- 11:00—Animal released and placed on the floor, lies on its side greatly prostrated.
- 11:05—Attempts to rise but falls back, unable to support itself on its legs; becoming progressively weaker.
- 11:50—Appears to be extremely hyperexcitable; picked up and then put back on the floor again; immediately had a convulsion with opisthotonus, coarse generalized tremors and drooling salivation. Lasted 3 minutes.  
The animal was then put back on the board and 25 cc. of calcium lactate saturated at room temperature, was injected intravenously. Convulsions and tremors immediately ceased. When the animal was released and placed on the floor, it arose in a few moments and was able to hop about. Animal was watched for 2 hours. Had no convulsions or tremors, nor appeared hyperexcitable, in spite of frequent stimulation. Animal remained alive and appeared well.  
Total P injected = 233 mgs.  
P per kilo injected = 106.2 mgs.

*Experiment 5.* Sept. 19. Weight of rabbit, 1760 gms.

Solution N NaOH.H<sub>3</sub>PO<sub>4</sub>, pH 6.4.

Uranium acetate titration = 5.24 mg. P per cc.

- 10:15-11:35—Injected 51 cc.  
During the injection the animal struggled and shivered a bit, but showed no tremors, convulsions, or salivation. Pulse rose from 100 to 190, respirations from 60 to 180.



When released, animal appeared very weak, and remained sprawled out for about one minute. Then it rose and hopped away. No further symptoms noted.

Total P injected = 267.6 mgs.

P per kilo injected = 152.1 mgs.

*Experiment 3.* Sept. 13. Weight of rabbit, 2000 gms.

Solution N NaOH.H<sub>2</sub>PO<sub>4</sub>. pH 5.6.

Uranium acetate titration = 5.56 mg. P per cc.

11:25-11:55—Injected 48 cc.

Animal showed no change in behavior except for elevation of pulse and respiration until just shortly before it was released when it began to shiver and to show a few fine tremors of the forelimbs. Upon being released, it lay sprawled out on the floor for a few moments and then rose and hopped away. No changes noted under constant observation during next 2 hours.

Total P injected = 226 mgs.

P per kilo injected = 133 mgs.

Examination of Table I shows these results in outline form. All animals which received alkaline phosphates and developed tetany invariably died within 24 hours; unless calcium lactate was administered intravenously, in which case they made a remarkably rapid recovery. In general, the minimum amount of alkaline phosphate required to produce tetany was about 100 mg. per kilogram body weight. Much larger doses of acid phosphate could be injected without exciting tetany, although in a few cases, very large doses caused a few slight tremors, and in one case, typical tetany. However, the contrast between the effects of alkaline and acid phosphates is extremely marked.

A few experiments were done to see whether sodium hydroxide alone would produce tetany. The results were entirely negative. Aside from its effects on the cardio-respiratory systems, no other symptoms were observed.

A protocol of one of these experiments is given:

*Experiment 16.* Sept. 23. Weight of rabbit, 2000 gms.

Solution 4/5 N NaOH.

10:00-10:30—Injected 45 cc.

During the injection animal showed no shivering or restlessness. Respirations became very slow and shallow and heart feeble. When released, animal was able to rise and move about, but became progressively weaker and died in about an hour without any signs other than gradual respiratory failure.



TABLE I.  
Intravenous Injection of Sodium Phosphate.

No. of experiments	Date	Weight of rabbit	Solution injected	pH of solution	Amount injected	Period of injection	Total P injected	P per kilo injected	Result	REMARKS
1	1922	gms.			cc.	min.	mg.	mg.		
2	Sept. 2	1630	N.NaOH.H <sub>3</sub> PO <sub>4</sub>	1.6	30	25	230	144	No tetany	
15	Sept. 12	1700	"	4.4	40	25	226	132	"	Great prostration. Few fine tremors. Made complete recovery.
	Oct. 19	1800	"	4.4	55	50	311	173	"	Few fine tremors.
3	Sept. 13	2000	"	5.6	48	30	266	133	"	
14	Oct. 5	1900	"	5.6	45	30	247	130	"	
5	Sept. 19	1760	"	6.4	51	25	267	152	"	
7	Dec. 23	2100	"	6.4	45	30	235	112	Tetany	3 hours after injection, animal had typical tetanic convulsion. Recovered completely.
6	Sept. 1	2060	"	7.4	53	30	236	115	"	Died within 24 hours.
11	Sept. 20	1300	"	7.4	28	30	134	100	"	Injection of calcium lactate caused cessation of symptoms.
8	Sept. 9	2200	"	8.8	55	45	233	106	"	Symptoms relieved by calcium lactate injections.
12	Oct. 21	2250	"	8.8	54	40	225	100	"	Died within 24 hours.
9	Sept. 8	1700	"	10.0	50	60	168	100	"	Died within 24 hours.

The administration of potassium phosphates gave results similar to those of the sodium salts (see Table II). At first normal solutions were used, but owing to the extreme depressant action of the potassium ion, the animals died of cardiac and respiratory failure after a few cubic centimeters of the solution had been injected. The solutions were therefore made half the original strength and injected very slowly and cautiously (1 and 2 hours). Here again, the alkaline potassium phosphates excited tetany, while acid phosphates did not. In the case of the potassium phosphates, however, approximately 50 mg. P per kilogram body weight were sufficient to cause tetany, or in other words, about one-half the dose required of alkaline sodium phosphates.

*The Influence of Phosphate Injection upon Calcium.  
Content of Blood.*

The above experiments were repeated, this time for the purpose of observing the calcium changes in the blood. All the sodium phosphates were administered in amounts of approximately 100 mgs. P per kilogram body weight, while one-half this dose of potassium phosphates were employed. The results are shown in Table III. Calcium determinations were made shortly before and one hour after injection. In every case, a reduction of calcium was observed, the amount of reduction being dependent only on the amount of phosphate injected and not upon the pH of the solution employed. With approximately 100 mgs. P per kilogram in the case of sodium phosphates, there resulted an average calcium drop of about 3.6 mgs. per 100 cc. blood. With 50 mgs. P per kilogram in the form of potassium phosphate, there was an average reduction of 1.6 mg. calcium per 100 cc. blood; in other words about one-half that obtained by using twice the dose of sodium phosphate. In spite of the fact that calcium reductions were obtained in all cases, only the alkaline phosphates produced tetany.

*The Influence of Other Alkaline Solutions Upon the Production  
of Tetany and the Calcium Content of the Blood.*

It next seemed desirable to inject alkaline solutions other than phosphates (still employing the same cation) to observe whether or not tetany could be produced, and if so, whether or not there occurred a similar reduction of the calcium ion. Sodium and

*Journal of Metabolic Research*  
Vol. 3

## ERRATA.

In the paper of Underhill, Gross and Cohen, this Journal, Vol. the following corrections should be made:

Page 690, two sentences should read as follows:

"With approximately 100 mg. P per kilogram in the case of sodium phosphate, there resulted an average calcium drop of about 8 mg. per 100 cc. blood. With 50 mg. P per kilogram in the form of potassium phosphate, there was an average reduction of 0.8 mg. calcium per 100 cc. blood."

Page 691, Table III should read:

## CALCIUM IN MG. PER 100 CC. BLOOD

Before injection		After injection		Reduction in calcium per 100 cc.	
mg.		mg.		mg.	
5.0		3.3		1.7	
5.2		3.1		2.1	
4.7		3.2		1.5	
4.5		2.6		1.9	
4.5		3.9		0.6	
4.9		4.0		0.9	
4.6		3.8		0.8	
No. of experiments		Weight		Date	
19		gm		1922	
		182		Oct. 11	
21		210		Oct. 16	
22		140		Oct. 18	
18		132		Oct. 10	
20		140		Oct. 13	
23		140		Oct. 19	
No. of experiments		Weight		Date	
25		8		1922 Dec. 2	
27		2		Dec. 23	
28		2		Dec. 26	
29		1		Dec. 24	
		1		1923	
30		2		Jan. 1	
31		1		Jan. 6	
32		1		Jan. 8	

potassium bicarbonate were the solutions chosen, but the latter was found too difficult to administer on account of its extreme depressant action on the heart. With the former too, very great difficulty was experienced but some results were obtained. The protocols are as follows:

*Experiment 35.* Feb. 17. Weight of rabbit, 2800 gms.

Solution 5%  $\text{NaHCO}_3$ .

3:30—Sample of blood collected for calcium determination.

3:40-4:20—Injected 80 cc. of solution.

The solution had to be injected very slowly, since respirations decreased markedly, the heart became slower and very weak, and at times periods of apnoea occurred. In spite of all precautions, the animal finally went into a state of apnoea and died without any tremors, twitching or convulsions.

Another sample for calcium determination was taken immediately upon the death of the animal by opening the thorax and aspirating some blood from the right auricle.

Total calculated amount of Na injected = 1095 mgs.

Amount of Na per kilo injected = 391 mgs.

Ca per 100 cc. blood before injection = 9.8 mgs.

Ca per 100 cc. blood after injection = 10.3 mgs.

*Experiment 36.* Feb. 20. Weight of rabbit, 2660 gms.

Solution 5%  $\text{NaHCO}_3$ .

12:00-12:50—Injected 40 cc.

1:00-1:50—Injected 41 cc.

During the first 5 minutes, respirations became deep and rapid (140), the pulse also increasing in rate. Then respirations slowed down gradually to 40 per minute and became very shallow; heart also became less rapid and weaker.

12:50—Heart slow and feeble. Respirations 15, very shallow. Injections stopped.

1:00—Injections continued, very slowly and cautiously.

1:30—Animal began to shiver and show tremors in forelimbs.

1:40—Tremors more generalized, beginning to salivate. Respirations increased to 80, heart rate 160.

1:50—Animal had a generalized clonic convulsion lasting 2 minutes, salivation more marked, cried. Injection stopped.

Sample of blood collected for calcium determination. Respirations gradually decreased in depth and rate; heart became weaker, and at 2:05 animal died of cardiac and respiratory failure without a terminal convulsion.

Total time of injection = 100 minutes.  
Total Na injected = 1108 mgs.  
Na per kilo injected = 416 mgs.  
Ca before injection = 9.2 mgs. per 100 cc. blood.  
Ca after injection = 9.8 mgs. per 100 cc. blood.

Examination of the protocols show that no drop in blood calcium occurred, but on the contrary, a slight increase. The symptoms produced were also by no means as marked or as typical of tetany as those obtained with phosphates. Death in both instances was due to cardiac and respiratory failure, and without severe terminal convulsions as noted with phosphates.

#### EXPERIMENTS WITH DOGS

Page 692, Experiment 35 should read:

Ca per 100 cc. blood before injection = 4.9 mg.  
Ca per 100 cc. blood after injection = 5.1 mg.

Page 693, Experiment 36 should read:

Ca before injection = 4.6 mg. per 100 cc. blood.  
Ca after injection = 4.9 mg. per 100 cc. blood.

can be accounted for either in the urine or blood. According to the theory of Tisdall, this sodium has passed out into the tissues. Due to the death of the animal it was impossible to obtain a 24 hour sample following injection. However, in the next table it will be seen that nearly all of the sodium and phosphorus injected can be accounted for by excretion through the urine in the 24 hours following injection.



TABLE IV.

Intravenous Injection of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ .  
Dog. 1. Female. Weight, 7.7 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	REMARKS
Mgs. 300 299	per 100 gms. 2.51 8.17	of whole blood 6.1 3.8	29.7 30.9	304.0 309.4	2.6 2.9	Normal 11:00 A. M. 1:30 P. M. injected intravenously with 250 cc. of 5.1 per cent solution of ortho-sodium phosphate. 2:30 P. M. salivation and nausea. 3:00 P. M. distinct facial tremors. 3:30 P. M. marked twitchings. 4:00 P. M. marked tetany. Blood sample. Dog recovered.

Calculations based on formula.

TABLE V.

Intravenous Injection of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ .  
Dog 2. Male. Weight, 12.5 kilos. Fasted 24 hours.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	REMARKS
Mgs. 306 301	per 100 gms. 2.94 11.7	of whole blood 5.9 4.1	27.8 29.2	321 326	2.9 3.2	Normal 10:15 A. M. 2:30 P. M. injected intravenously with 430 cc. of 5.1 per cent $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ . 3:45 P. M. twitching, salivation. 4:00 P. M. pronounced tetany. 4:15 P. M. blood sample. Dog dead next day.

Calculations based on formula.

This demonstrates that very little is lost by way of the intestinal tract.

The intravenous injection of the acid sodium phosphate failed to produce tetany in dogs when administered in concentration of equivalent phosphorus of the alkaline phosphate. The calcium and inorganic phosphates, however, were disturbed to about the same extent as with the disodium phosphate. There always appeared to be a slight diminution of sodium following injection of the primary sodium phosphate, but this change is too slight to be of any significance. The above table is characteristic of a number of experiments.

When the acid phosphate is given in a concentration of approximate sodium equivalent of the alkaline phosphate, fully as marked tetany occurs as with the secondary phosphate, with practically the same changes in the inorganic ions. This corroborates the findings of Greenwald that tetany may be produced by both the acid and alkaline phosphates.

Furthermore, the subcutaneous injection of calcium chloride failed either to allay the symptoms or to prolong life in dogs. It is quite evident from these experiments that low calcium is not the *primary* cause of tetany.

#### *The Effect of Injection of Primary and Secondary Potassium Phosphate.*

Attempts to administer the potassium phosphates intravenously were unsuccessful, so that they were injected in all cases intraperitoneally.

With the intraperitoneal injection of the secondary potassium phosphate marked tetany was manifested. When calculated on the phosphorus basis, it requires only about  $\frac{2}{3}$  the concentration of the potassium salt as the sodium salt to produce tetany. However, when these salts are calculated on the amount of base administered the concentrations are of nearly equal strength.

Here again no marked disturbances are shown except in the cases of calcium and the inorganic phosphates. The changes in calcium on the average were somewhat less than the sodium salts, probably due to the fact that less phosphate radicle was injected.

An interesting result following the intraperitoneal injection of isotonic potassium phosphate solutions was the marked blood concentration, as determined by the increase in hemoglobin.

TABLE VI  
Intravenous Injection of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$   
Dog 3. Female. Weight, 7.35 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	Sodium in urine	Phosphorus in urine	REMARKS
302	Mgs. 3.6	per 100 gm. 5.7	s. of whole 30.1	blood. 312	3.2	gms. .251	gms. .203	Normal. 11:50 A. M. injected intravenously with 250 c.c. of 5.1 per cent solution of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ . 1:30 P. M. dog in tetany. Blood sample and urine collected. Dog dead at 5 P. M.
292	6.3	3.45	31.4	320	3.0	1.103	.866	

TABLE VII  
Intravenous Injection of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$   
Dog 4. Female. Weight, 7.1 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	Sodium in urine	Phosphorus in urine	REMARKS
298	Mgs. per 2.9	100 gms. 6.05	of whole blood. 27.4	319	2.8	gms. .243	gms. .248	Normal. 11:45 A. M. injected intravenously with 240 c.c. of a 5.1 per cent solution of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ . 12:15 P. M. nausea and salivation. 12:30 P. M. twitching. 1:00 P. M. marked twitching. Blood sample, 24 hour urine sample. Dog recovered.
304	8.4	4.10	30.0	323	3.0	1.663	1.217	

TABLE VIII.

Intravenous Injection of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ . Dog 5. Male. Weight, 9 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	REMARKS
	Mgs. per 100 gms.		of whole blood			
307	2.45	5.6	28.2	324	3.1	Normal.
304	6.2	4.2	29.4	313	3.0	1:30 P. M. injected intravenously with 270 c.c. of 2.34 per cent $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ .
						2:30 P. M. no symptoms except salivation and nausea.
						3:00 P. M. no symptoms. Blood sample.
						4:00 P. M. no symptoms.
						Dog recovered.

Calculations based on formula.

TABLE IX.

Intravenous Injection of Phosphates. Dog 6. Male. Weight, 13.3 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	REMARKS
	Mgs. per 100 gms.					
303	2.46	of whole blood	30.2	341.5	3.1	Normal 11:00 A. M.
310	13.20	6.00	29.2	332.0	2.7	*Injected intravenously with 17.7 gms. of monosodium phosphate in 325 c.c.
		3.70				2:00 P. M. began injection.
						2:15 P. M. injection complete.
						3:00 P. M. dog showed tremors, salivation and nausea.
						3:30 P. M. hind legs paralyzed with distinct tremors in neck and front legs.
						3:45 P. M. quite marked tetany.
						Blood sample taken.
306	9.80	5.8	31.4	335	3.3	3:40 P. M. injected subcutaneously with 15 c.c. of 5 per cent calcium chloride.
						4:30 P. M. tetany unchanged.
						5:00 P. M. tremors, very restless. Blood sample.
						6:00 P. M. dog slightly quieter with labored breathing.
						8:00 P. M. dog dead.

\*Not given in isotonic solution. Calculations based on formula.

TABLE X.  
Intravenous Injection of Phosphates  
Dog. 7. Male. Weight, 12.5 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	REMARKS
Mgs. per 100 gms. of		whole blood				
306	3.1	5.9	31.2	327	2.6	Normal 9:20 A. M.
301	15.6	3.3	30.7	316	3.0	*Injected with 16.6 gms. of mono-sodium phosphate in 30 c.c. water, at 12:10 P. M.
						1:00 P. M. slight tremors, marked salivation and nausea.
						1:15 P. M. distinct general tetany.
						1:30 P. M. tetany marked. Blood sample.
304	10.1	6.2	29.6	Lost	2.8	1:40 P. M. injected subcutaneously 15 c.c. of 5 per cent $\text{CaCl}_2$ solution.
						2:30 P. M. tetany still persistent. Blood sample.
						3:00 P. M. dog in coma.
						3:10 P. M. dog dead.

\*Not given in isotonic solution.

TABLE XI  
Intraperitoneal Injection of Phosphates  
Dog. 8. Male. Weight, 12 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	Hemoglobin	REMARKS
	Mgs. per 100 gms. of		whole blood			per cent	
305	2.9	5.9	28.7	311.3	2.9	96	Normal 10:30 A. M.
270	10.5	4.3	33.0	304.0	2.7	97	2:15 P. M. given 250 c.c. of 2.7 per cent $\text{K}_2\text{HPO}_4$ intraperitoneally.
						112	3:30 P. M. dog nauseated and depressed.
						119	4:00 P. M. symptoms of tetany.
						126	4:30 P. M. very distinct twitchings, head movement and teeth chattering.
							5:20 P. M. dog dead.

Calculations based on formula.



Blood concentrations of 25-35 per cent. frequently followed such injections. These blood concentrations also occurred after the intraperitoneal injection of the sodium phosphates.

*Effect of the Acid Potassium Phosphate.*

When the acid potassium phosphate was injected in equivalent of phosphorus of the secondary phosphate no tetany was manifested, although slight facial twitchings were perceptible. When the acid salt was increased about  $\frac{1}{2}$  then marked tetany occurred, demonstrating again that it is the amount of base present which is the determining factor in the production of tetany with the phosphates.

*The Influence of Phosphate Injections Upon the Composition of the Bones of Rabbits.*

Several experiments were conducted to determine whether or not any reduction occurred in the calcium content of the bones of rabbits which had received phosphates. Although not originally planned as part of the investigation, these experiments were suggested by the fact that three of the rabbits which had been injected with acid phosphates and returned to their cages, shortly afterward were found to have fractured one or more of their legs. One rabbit, when accidentally dropped from a height of about 9 inches to the floor of its cage, fractured both forelegs and one hindleg. These occurrences seemed to be more than mere coincidences. Inasmuch as reduction in blood calcium was known to occur following the intravenous injections of phosphates, it was thought that a rarefaction of the bones might have resulted through the attempt to restore tissue equilibrium by drawing upon this source of supply of calcium. In this connection it is worthy of note that Erdheim<sup>40</sup> found in his experiments on the production of tetany in rats, that most extraordinary changes occurred in these animals' teeth, which became fragile, opaque, and frequently broke off short, indicating a disturbance in the calcium metabolism.

In these experiments, acid sodium phosphates were used, since larger amounts could be injected, thereby causing a greater reduction of calcium without producing death. The hind limbs of the rabbits were X-rayed before injection and at various intervals thereafter. At the end of the period of observation, the animals

TABLE XII  
Intraperitoneal Injection of Phosphates  
Dog. 9 Female. Weight, 9 kilos.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	Hemoglobin	REMARKS
310	Mgs. per	100 gms. of	whole blood			per cent	
299	2.5	6.3	31.1	306	3.0	102	Normal 11:30 A. M.
	16.1	4.4	35.3	316	2.8	101	2:10 P. M. injected 185 c.c. of 2.7 per cent $K_2HPO_4$ intraperitoneally.
						111	2:40 P. M. symptoms of tetany.
						119	3:00 P. M. marked tremors and twitching.
						129	3:20 P. M. tetany. Blood sample.
							4:45 P. M. dog dead.

Calculations based on formula.

TABLE XIII  
Intraperitoneal Injection of Phosphates  
Dog. 10. Female. Weight, 11.5 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	Hemoglobin	REMARKS
	Mgs.	per 100 gms.	of whole blood.			per cent	
298	3.1	6.05	27.6	322	3.0	99	Normal 10:40 A. M.
304	7.1	4.40	31.9	319	3.3	47	2:00 P. M. injected 210 c.c. of 2.4 per cent $KH_2PO_4$ intraperitoneally.
						115	2:30 P. M. nausea and depression, diarrhoea.
							No tetany.
						119	3:00 P. M. probably very slight tremors.
						122	3:30 P. M. tremors disappeared, blood sample.
							5:45 P. M. dog dead.

Calculations based on formula.

were killed, and the calcium content of the dehydrated cortex of the bones of the hind limb (femur, tibia, and fibula) was determined. Calcium determinations of the bone ash were made according to McCrudden's method.<sup>47</sup>

The bones of two normal rabbits were first analyzed to determine the normal per cent. of calcium in bone ash (see Table XV). This was found to be approximately 50 per cent. All determinations in the above and the following experiments were done in duplicate, that is both hind limbs were used.

*Experiment C.* Weight of rabbit, 1950 gms.

Oct. 6—Animal X-rayed; then injected with a solution of  $\text{N.NaOH.H}_2\text{PO}_4$ , pH 5.6; receiving 135 mgs. P per kilo. No tetany developed.

Oct. 13—X-ray showed no change in bone shadow.

Oct. 18—X-ray again showed no obvious change.

Animal killed and bones analyzed.

Result—Ca in ash = 49.09 per cent.

*Experiment D.* Weight of rabbit, 2100 gms.

Oct. 23—Animal X-rayed and then injected with a solution of  $\text{N.NaOH.H}_2\text{PO}_4$ , pH 4.4; 173.3 mgs. P per kilo.

Nov. 1—X-ray showed no change.

Nov. 2—Animal again injected with same solution, 120 mgs. P per kilo.

Nov. 8—X-ray showed no change in bone shadow.

Nov. 9—Animal killed and bones analyzed.

Result—Ca in bone ash = 50.86 per cent.

*Experiment* (Rabbit E, weight 2250 gms.)

(Rabbit F, weight 1860 gms.)

Nov. 10—Both animals placed in cages and given only water.

Nov. 13—Rabbit F received intravenously 140 mgs. P per kilo body weight in the form of sodium phosphate, pH 6.4. Then returned to cage and again kept on water regime.

Nov. 15—Both animals killed and bones analyzed for calcium.

Result—(Rabbit E. Ca in ash = 51.77 per cent.)

(Rabbit F. Ca in ash = 50.83 per cent.)

The results of the above experiments are shown in tabulated form in Table XV. Examination of this table shows that there are negligible variations in the per cent. of calcium in the ash of all bones analyzed. In experiment C, there is a wider variation in the per cent. of ash and calcium in the dry cortex, but even these are within the limits of experimental error. The conclusion

TABLE XIV  
Intraperitoneal Injection of Phosphates  
Dog 11. Female. Weight, 8 kilos. Fasted 24 hours before experiment.

Chlorides	Inorganic Phosphates	Calcium	Potassium	Sodium	Magnesium	Hemoglobin	REMARKS
311	Mgs. per 100	gms. of	whole blood.			per cent	
307	2.8	5.7	30.7	334	2.4	103	Normal 11:30 A. M.
	11.7	3.9	33.6	327	2.6	101	2:30 P. M. injected 220 c.c. of 2.4 per cent $\text{KH}_2\text{PO}_4$ intraperitoneally.
						109	3:00 P. M. salivation, nausea, heart very irregular.
						123	3:30 P. M. distinct tremors.
						128	3:45 P. M. distinct tetany. Blood sample.
							4:30 P. M. dog dead.

Calculations based on formula.

TABLE XV  
Effect of Phosphate Injections on Bone Calcium

Rabbit	Wt. of bone cortex before dehydration	Wt. of bone cortex after dehydration	Wt. of water removed	Wt. of ash	Per cent Ash in dry bone cortex	Wt. of Calcium	Per cent of calcium in ash	Per cent of calcium in dry bone cortex
	gms.	gms.	gms.	gms.				
A. Normal	6.4540	5.3033	1.1507	3.1839	60.0	1.5967	50.15	30.1
B. Normal	7.1700	5.2459	1.9241	3.2716	62.3	1.6178	49.45	30.8
C. After injection	7.0767	4.6355	2.3912	2.6401	56.3	1.2962	49.09	27.6
D. after injection	10.0677	5.9206	4.1471	3.5371	59.7	1.7992	50.86	30.3
E. Control	12.1872	8.6622	3.5250	5.1472	59.4	2.6651	51.71	30.8
F. After injection	10.7912	7.6692	3.1220	4.7230	60.2	2.4006	50.83	31.1

drawn from these experiments is that under our experimental conditions intravenous injection of sodium phosphates fails to produce quantitative changes in the calcium content of bone cortex.

### *Discussion.*

The question arises as to how phosphates injected either intravenously or intraperitoneally are concerned in the production of tetany. It is obvious that a reduction in blood calcium, per se, is not sufficient to produce tetany, since this failed to occur with the lower concentrations of the acid phosphates, in spite of the accompanying calcium decrease. Furthermore calcium chloride injections in dogs was practically ineffectual in either stopping the tetany or prolonging the animals life, in rabbits it apparently prevented death. Also following intravenous injections of sodium bicarbonate in rabbits convulsions occur and yet there is no demonstrable reduction of calcium.

The theory of alkalosis is untenable due to the fact that the acid phosphates will produce tetany. Furthermore, studies by Tisdall<sup>48</sup> and by Greenwald<sup>32</sup> have shown that a condition of alkalosis does not follow even after the injection of the alkaline sodium phosphates.

Investigations into the etiology of tetany, of late have been directed not only toward observing the quantitative changes in calcium, but also changes in the amounts of other inorganic constituents in the blood, and particularly their quantitative relationship to each other. This attitude is based chiefly on the work of Loeb and others who have shown that the maintenance of normal irritability of muscle and nerve tissue is dependent upon an undisturbed equilibrium between various electrolytes in the surrounding medium, and that certain divalent cations are antagonistic to monovalent cations in their influence upon nerve excitability. In general, monovalent cations are stimulating, while divalent cations are inhibiting. Loeb<sup>1</sup> regarded the Na and Ca ions as especially antagonistic, and stated that both had to be present in proper proportions for the maintenance of a physiological balanced solution. In 1908, Fuhner<sup>49</sup> suggested the possibility of an increase in the ratio  $\frac{\text{monovalent ions}}{\text{divalent ions}}$  as the cause of



tetany. Finally Loeb<sup>50</sup> found that to preserve normal irritability, the ratio  $\frac{\text{Na} + \text{K}}{\text{Ca} + \text{Mg}}$  must be maintained fairly constant, and that the amount of calcium required to neutralize sodium varies in direct proportions to the concentration of the latter. While it has been generally conceded that the cations are the more important elements, recent investigations have tended to show that the anions are not entirely devoid of action. Raber,<sup>51</sup> who conducted researches on the effect of anions on the conductivity of living protoplasm, concluded that the anions have a definite effect on the chemical stimulation of nerves, although to a less degree than the cations. The monovalent anions are inhibiting, while the divalent anions are exciting; in other words, the exact opposite of the action of the cations.

Theoretically, therefore, one would expect a salt with a monovalent cation and a divalent anion to be the most stimulating. However, this view cannot be accepted too literally. Thus, barium, a divalent cation and theoretically depressant, when injected intravenously causes violent tonic and clonic spasms, from its stimulating the spinal cord and medulla oblongata. Also the extreme depressant effect of magnesium salts is strikingly counteracted by calcium which appears to be specific in this respect; in spite of the fact that calcium is depressant. Hastings and Murray called attention to the work of Munzer in support to the theory presented above. In 1898, Munzer<sup>52</sup> studied the salt action of various sodium salts when injected intravenously into rabbits, working with the chloride, iodide, nitrate, sulphate, acetate, bicarbonate, and phosphate. He found that all caused convulsions and produced death if given in proper concentration and if injected at the proper rate. Investigations into the minimal lethal dose of these salts per kilogram body weight showed the sodium phosphate to be the most toxic, and the sodium bicarbonate next in toxicity. These results are in accord with the theory outlined above, since greatest toxicity was possessed by salts with monovalent cations and polyvalent anions. Greenwald<sup>53</sup> obtained practically similar results with the injection of sodium salts, except that in his hands, sodium chloride was the most toxic. He attributed the symptoms to a "disturbance in the relation between sodium and potassium, calcium and possibly

other ions in the blood and alter tissues," and also to the "nature of the anion but probably only through its effect in determining the permeability of the cells to sodium." He has repeatedly maintained that the phosphate ion is devoid of any toxic action, and that tetany following the injection of sodium phosphate is due chiefly to sodium poisoning.

The theory of sodium poisoning, or in our experiments, both sodium and potassium, appears to be the most tenable theory at present. The tetany produced by us in dogs, apparently was dependent upon the amount of base administered. While, we were unable to demonstrate any increase of base in the blood stream, we believe that the basic ions exert their action in the tissues themselves. Whether the tetany produced by bases is peripheral or central has not been determined.

If the base passes to the tissues, then undoubtedly there is a disturbance in the ionic equilibrium of the tissues themselves that produce the tetany. The nature of this disturbance is unknown.

The theory of Tisdall of the action of the alkaline phosphate versus the acid phosphates is untenable from our own results. He found, among other things, that following the injection of alkaline sodium phosphates in dogs, there resulted a reduction of calcium from an average of 10.5 mg. per 100 cc. to 6.2 mg., and no definite change in the sodium content of the serum. On the other hand, when phosphoric acid was employed, he not only obtained a reduction of calcium, but also a decided reduction of the sodium of the serum. In both instances, the potassium remained constant, while the inorganic phosphorus increased from about 5.0 mg. to an average of 18.0 mg. He attributed the tetany following the injections of diacidic sodium phosphate,

therefore to a change in the  $\frac{\text{Na}}{\text{Ca}}$  ratio. With phosphoric acid,

this ratio did not change, because accompanying the calcium reduction, there was a simultaneous diminution of the sodium in the processes of neutralization and excretion of the injected acid.

The  $\frac{\text{Na}}{\text{Ca}}$  ratio, therefore, according to Tisdall, is the important factor in the production of tetany. On the same basis, tetany

following hyperpnea results from an increase in  $\text{NaHCO}_3$ , and therefore irritating sodium salts in the tissue.

Calculations based on Tisdall's figures, however, are not so convincing. The normal  $\frac{\text{Na}}{\text{Ca}}$  ratio in all animals employed was fairly constant, the average value being 32.8. In those animals which had received dibasic phosphate with resulting evidences of toxæmia, the  $\frac{\text{Na}}{\text{Ca}}$  ratio rose to an average of 56.4. On the other hand, in those animals which had received phosphoric acid without any disturbances, the average  $\frac{\text{Na}}{\text{Ca}}$  ratio was only slightly less; namely, 50.5. In some individual cases, the  $\frac{\text{Na}}{\text{Ca}}$  ratio in the second group was as great and even greater than those in the first group. It hardly seems plausible, therefore, that this ratio alone is the essential factor. From the fact that acid phosphates do produce tetany, make this theory impossible.

Kramer, Tisdall, and Howland,<sup>39</sup> in studying the changes occurring in the blood of infants with infantile tetany, found that the ratio  $\frac{\text{Na} + \text{K}}{\text{Ca} + \text{Mg}}$  is increased in this condition. This they found to be due almost entirely to a reduction in the concentration of calcium. The concentration of Na and Mg was essentially normal, while that of K was slightly increased. Recently Gross and Underhill,<sup>54</sup> in a very inclusive study of the inorganic ion balance in parathyroidectomized dogs, found that the essential changes were an increase in potassium and a decrease in calcium of the serum. The sodium, magnesium, and chlorides remained unchanged, while the phosphorus was slightly increased in a few cases. When the  $\frac{\text{K}}{\text{Ca}}$  ratio increased from a normal of about 5 to 10 or above, convulsive tetany developed. Similarly, when the ratio of the  $\frac{\text{monovalent ions } (\text{Na} + \text{K})}{\text{divalent ions } (\text{Mg} + \text{Ca})}$  changed from a normal of 30 to 40 or above, convulsive tetany developed. They concluded, therefore, that parathyroidectomy disturbs chiefly the ratio between K and Ca and that tetany supervenes when definite changes in this ratio develop.

No such marked changes occur between calcium and potassium in the tetany produced by the phosphates. It is evident we are

dealing with another type of tetany, and that all tetanies cannot be ascribed to the same factors.

There is no direct evidence that the phosphate ion plays any specific role in the production of tetany following the intravenous injection of phosphates, except in so far as it may aid in causing a diminution in calcium and thus disturbing the normal ionic equilibrium. No constant changes in the concentration of inorganic phosphorus of the serum have been observed in infantile tetany, parathyroid tetany, or tetany following pyloric obstruction. Neither Tisdall<sup>48</sup> nor Gross and Underhill<sup>54</sup> were able to attach any significance to changes in the calcium phosphorus ratio. Similarly, the concentration of the chlorides and sulphur in the serum has been studied with varying results. In gastric tetany, however, a reduction in plasma chlorides is the most constant change observed, and a disturbance in ionic equilibrium from this cause may be fundamental in this type of tetany. This seems to be confirmed also by the fact that the intravenous injection of HCl, NaCl,  $\text{NH}_4\text{Cl}$ , or anything which supplies the missing Cl ion relieves the symptoms of tetany.

The study of the inorganic ion balance in tetany is still in its infancy. The results obtained thus far, however, have been very valuable and stimulating, and have pointed the way for further investigation. As yet, tetany cannot be ascribed to any one specific disturbance. It is important to preserve a broad attitude, for as Greenwald has put it, "any one of a multitude of disturbances in the equilibrium within certain tissues may be responsible."

### SUMMARY

1. Solutions of sodium and potassium phosphates when injected intravenously or intraperitoneally in dogs and rabbits cause a diminution of calcium, the degree of diminution being more or less dependent on amount of phosphate introduced.
2. Both the alkaline and acid phosphates of sodium and potassium produce tetany, at least in the dog.
3. The injection of calcium salts apparently stopped the tetany in rabbits, but was not beneficial in dogs.
4. The tetany produced was dependent on the amount of base introduced.

5. The intravenous administration of  $\text{NaHCO}_3$  produces tetany, but no drop in calcium.

6. Following intravenous injections of comparatively large doses of sodium ortho-phosphate, no quantitative changes in respect to calcium occur in the bones of rabbits, as determined by X-ray pictures and chemical analysis of the bones.

7. The intraperitoneal injection of phosphates causes a marked concentration of the blood which must be regarded as a factor contributing to death which usually results.

#### BIBLIOGRAPHY.

1. Loeb, J. *Am. J. Physiol.*, 3, 1900, 327.
2. Sabbatini, L. *Mem. Acad. Reale Sc. Torino*, 54, 1904, series 2, 459.
3. Wuest, R. *Jahrb. f. Kinderk.*, 61 Berlin, 114.
4. Cohn, M. *Deutsch. Med. Woch.*, 33, 1907, 1987.
5. Aschenheim, E. *Monatschr. f. Kinderk., Leipzig Wien.*, 9, 1910, 366.
6. Neurath, R. *Zeitschr. f. Kinderk.*, Berlin, 1, 1910, 1-42.
7. Cattaneo, C. *LaPediatri.*, Napoli, 1909, 7, 2nd series, 414. (Cited by MacCallum and Voegtlin.)
8. Longo, A. *Il Policlinico*, Rome, 17, 1910, 495. (Cited by MacCallum and Voegtlin.)
9. MacCallum, W. G., and Voegtlin, C. *J. Exp. Med.*, 11, 1909, 118.
10. MacCallum, W. G., and Vogel, K. M. *J. Exp. Med.*, 18, 1913, 618.
11. Marriott, W. McK., and Howland, J. *Arch. Int. Med.*, 18, 1916, 708.
12. Binger, C. *J. Pharm. and Exp. Therap.*, 10, 1917, 105.
13. Howland, J., and Marriott, W. McK. *Quart. J. Med.*, 11, 1917-18, 289.
14. Hastings, A. B., and Murray, H. A., Jr. *J. Biol. Chem.*, 46, 1921, 233.
15. Howland, J., and Marriott, W. McK. *Tr. Am. Ped. Soc.*, 28, 1916, 202.
16. Barach, A. L., and Murray, H. A., Jr. *J. Am. Med. Assn.*, 74, 1920, 786.
17. Underhill, F. P., and Saiki, T. *J. Biol. Chem.*, 5, 1908-09, 225.
18. Cooke, J. V. *J. Exp. Med.*, 13, 1911, 439.
19. Morel, L. *J. de Physiol. et de Pathgen.*, 13, 1911, 542.
20. Wilson, Stearns, and Janney. *J. Biol. Chem.*, 21, 1915, 169.
21. Wilson, Stearns, and Thurlow. *J. Biol. Chem.*, 23, 1915, 89.
22. Wilson, Stearns, and Janney. *J. Biol. Chem.*, 23, 1915, 123.
22. McCann. *J. Biol. Chem.*, 35, 1918, 553.
23. MacCallum, W. G., et al. *Bull. Johns Hopkins Hosp.*, 31, 1920, 1.
24. Grant, S. B. *Arch. Int. Med.*, 30, 1922, 355.
25. Collip, J. B., and Backus, P. L. *Am. J. Physiol.*, 51, 1920, 568.
26. Grant, S. B., and Goldman, A. *Am. J. Physiol.*, 52, 1920-21, 209.
27. Tileston, W. (Cited by Palmer and Van Slyke.) *J. Biol. Chem.*, 32, 1917, 499.
28. Harrop, G. A., Jr. *Bull. Johns Hopkins Hosp.*, 30, 1919, 62.



29. Togawa, T. *J. Lab. and Clin. Med.*, 5, 1919-20, 299.
30. Underhill, F. P., and Nellans, C. T. *J. Biol. Chem.*, 48, 1921, 557.
31. Hastings, Murray, and Murray. *J. Biol. Chem.*, 46, 1921, 223.
32. Greenwald, I. *J. Biol. Chem.*, 54, 1922, 285.
33. Paton, C. N., and Findlay, L. *Quart. J. Exp. Physiol.*, 10, 1916, 203.
34. Luckhardt, A. B., and Rosenbloom, P. J. *Proc. Soc. Exp. Biol., and Med.*, 19, 1921-22, 129.
35. Freudenberg, and Gyorgy. *Jahrb. f. Kinderk.*, 96, 1921, 5.
36. Meysenberg, and McCann. *J. Biol. Chem.*, 47, 1921, 541.
37. Greenwald, I. *J. Biol. Chem.*, 14, 1913, 369.
38. Jepson, and Klercker. *Ztschr. f. Kinderk.*, 28, 1921, 71.
39. Kramer, B., Tisdall, F. F., and Howland, J. *Am. J. Diseases of Children*, 22, 1921, 431.
40. Clark. "The Determination of Hydrogen Ions," Baltimore, 1920, pg. 69.
41. Underhill, F. P. "A Manual of Selected Biochemical Methods," pg. 192.
42. Kramer, B., and Tisdall, F. F. *J. Biol. Chem.*, 48, 1921, 223.
43. Whitehorn, J. C. *J. Biol. Chem.*, 45, 1921, 449.
44. Briggs, A. P. *J. Biol. Chem.*, 52, 1922, 13.
45. Hawk. "Practical Physiological Chemistry," 6th Edition, p. 576.
46. Erdheim. *Sitz. d. k. Acad. d. Wiss, Wien. Math. Naturwiss, kl.*, 116, 1907, iii, 311. (Cited by MacCallum and Voegtlin.)
47. Hawk. "Practical Physiological Chemistry," Philadelphia, 1916, pgs. 560-61.
48. Tisdall, F. F. *J. Biol. Chem.*, 54, 1922, 35.
49. Fuhner, H. *Archiv. Exp. Path. u. Pharmacol.*, 58, 1907-08, 1. (Cited by Hastings and Murray, 14.)
50. Loeb, J. *J. Biol. Chem.*, 23, 1915, 423.
51. Raber, O. L. *J. Gen. Physiol.*, 2, 1919-20, 535-541.
52. Munzer, E. *Archiv. Exp. Path. u. Pharmacol.*, 41, 1898, 74.
53. Greenwald, I. *J. Pharm. and Exp. Therap.*, 11, 1918, 281.
54. Gross, E. G., and Underhill, F. P. *J. Biol. Chem.*, 54, 1922, 105.



## EFFECT OF RATIONS CONTAINING WHOLE AND SKIMMED MILK ON YOUNG GROWING PUPPIES.

MARGUERITE DAVIS.

*From the Home Economics Laboratory, University of Wisconsin.*

*Pathological Examination by Paul F. Clark.*

*Department of Pathology, University of Wisconsin.*

That diet can produce changes in the tissues is beyond question. With some diets these changes are definite, while with others and particularly with those deficient to a mild degree the nature of the changes is dependent upon the conditions under which the experiment is carried out. The metabolism is influenced by many factors and it is impossible to keep all of the factors save the diet uniform. It would thus seem that only by the slow accumulation of experiments carried out under different conditions can the role of the mildly deficient ration in the production of pathological conditions be determined.

The experiments here reported deal with a type of ration which is declared by some investigators to produce rickets, while others contend that diet is not the chief factor and that this particular type does not invariably lead to rickets. Much of the controversy arises from the fact that rations which contain different amounts of skimmed milk are treated as comparable while as a matter of fact they differ not only in vitamin content but also in the composition of the inorganic and protein constituents of the ration which in turn affect the susceptibility to vitamin deficiency.

Another source of confusion is the fact that not only are experiments with different kinds of animals compared but also experiments with animals that are infested with parasites and are suffering from intestinal disturbances are compared with experiments in which the animals are free from such disturbances.

Both Powers, Park, Shipley, McCollum and Simmonds,<sup>1</sup> and Hess, Unger and Pappenheimer<sup>2</sup> have shown that the amount of exposure to sunlight is a very important factor in feeding experiments as sunlight has the same effect on bone growth as vitamin A. Space does not permit of a consideration of further variable conditions.

## EXPLANATION OF EXPERIMENTS.

Our experiments were based on those of Mellanby.<sup>2</sup> He concluded that the following conditions inhibit calcification or increase growth relatively to calcification so that defectively calcified bone results:

- (1) A deficiency of calcium and phosphorus in diet.
- (2) A deficiency of fat containing the anti-rachitic vitamin.
- (3) Excess of bread, other cereals, and carbohydrates.
- (4) Absence of meat.
- (5) Excess of protein moiety of casein free from calcium.
- (6) Lack of exercise.

The conditions of our experiments have been rather more extreme than those of Mellanby. The age of our puppies at the beginning of the experiment ranged from four to six weeks with an average of five weeks, against six to nine weeks in Mellanby's experiments. The initial weight of the puppies corresponds closely in the two cases; the range for our puppies is from 850 to 1850 grams, with an average of 1300, while Mellanby reports a range of 840 to 2410 gms.

## RATIONS.

In the earlier experiments of Mellanby the following ration gave rise to rickets after a long period often four to six months:

Whole milk 200 cc.  
Oatmeal and rice in equal parts.  
Salt.

Increase in milk to  $\frac{1}{2}$  liter daily prevented rickets while the substitution of skimmed milk for whole milk resulted in the rapid development of marked rickets.

Our experiments were based on these early ones of Mellanby. The purpose was to compare whole and skimmed milk. The rations were as follows:

Whole milk 100 cc.	Skimmed milk 100 cc.
Oatmeal.	Oatmeal.
Salt.	Salt.

In the first experiment the rations were:

Skimmed milk 200 cc.	Condensed milk 40 grams.
Oatmeal.	Oatmeal.
Bread.	Bread.
Salt.	Salt.

40 grams of condensed milk corresponds to 120 to 150 cc. of fresh milk.

The skimmed milk was obtained each morning from the University Dairy barn. It was the morning's milk and was separated by machine and not pasteurized. The fat content did not exceed .1 per cent. The whole milk was delivered each morning by a local dealer. It was pasteurized.

The milk was the sole source of antiscorbutic vitamin in the ration. There was no sign of scurvy in any of the puppies. If subacute scurvy did exist it would doubtless be greater on the whole milk ration because that was pasteurized.

We have compared two rations incomplete to different degrees. This was done because we wanted one ration to be extremely deficient and the other to resemble it in all respects but one; i. e., the fat content of the milk.

#### CALCIUM AND PHOSPHATE IN RATION.

The ash analysis of whole and skimmed is given as follows:<sup>4</sup>

	Ca	Mg	K	Na	P	Cl	S	Fe
Whole milk	.120	.012	.143	.051	.093	.106	.034	.00024
Skimmed milk	.122	.012	.149	.052	.096	.110	.035	.00025

The important differences between the whole and the skimmed milk are, therefore, so far as our present knowledge goes, those of fat and the vitamin carried by the fat.

#### DESCRIPTION OF EXPERIMENT.

The puppies were a mixture of French bull and daschund and were bred in the laboratory of the Genetics Department. They were weaned as soon as they were able to eat from a dish and were put on ration at five weeks.

As they grew older they became lively, always anxious to play and very impatient for their milk. The undersized puppies were not as active or as friendly as the larger ones. They were not undersized as a result of the ration but due to the condition in which they were received in the laboratory.

The puppies were kept in a large runway in a well-aired laboratory that received the morning sun. Our experience has been that animals eat better when they are fed together, so they were given a common dish of oatmeal. For the milk they were taken from the runway and fed separately.

The amount of oatmeal consumed was influenced by the temperature and the previous condition of the puppy. In cool weather the appetite was good until a marked degree of malnutrition had developed.

Those that received the whole milk grew for a longer period than those that received the skimmed milk. Increase in weight is not an accurate index of increase in stature as the puppies that received the skimmed milk were in some cases thinner than the others. The individual variation in the rate of growth was greater than the group variation. This is in accord with Mellanby's observation that the rate of growth was the same for 10 weeks on the adequate and inadequate diets. After 10 weeks he found that less food was consumed on the inadequate diet.

In the preliminary period of the first experiment puppies 1, 2 and 3 received skimmed milk once a day in a common dish. After six weeks on the experiment Dog 1 was killed for examination. In addition to the external symptoms of pot-belly and beaded rib, the structure of the costo-chondral junction was found to be abnormal. Dogs 2 and 3 were



from then given their skimmed milk once a day separately and received 200 cc. each. This was not far from the amount that they had been receiving.

Six weeks later Dog 2 was unable to use his hind legs and was in such a helpless condition that he was killed. Dogs 1 to 4 were of the same litter. Dog 3 was a brindle and did not grow as rapidly as the three black dogs. Although markedly humpbacked and pot-bellied, he did not lose the use of his legs. This is in accord with Mellanby's observation that the rapidly growing puppies showed the most extreme symptoms.

Dog 4 received 40 grams of condensed milk daily.

TABLE I.  
PRELIMINARY PERIOD OF FIRST EXPERIMENT

Date 1920	No. of Animal	Age in Days		Ration	Weight in gms.			Remarks
		Initial	Final		Initial	Max.	Final	
Jan. 28	1	31	73	Skimmed milk, oatmeal, bread	1690	3475	3475	Killed for examination
Jan. 28	2	31	73	Skimmed milk, oatmeal, bread	1323	3490	3490	Continued in Table II.
Jan. 28	3	31	73	Skimmed milk, oatmeal, bread	991	2170	2170	Continued in Table II.
Jan. 28	4	31	73	40 gms. cond. milk, oatmeal, bread.	1538	3255	3255	Continued in Table II. Without change in ration.

TABLE II.  
LATER PERIOD OF FIRST EXPERIMENT

Date 1920	No. of Animal	Age in Days		Ration	Weight in gms.			Remarks
		Initial	Final		Initial	Max.	Final	
Mar. 11	2	74	116	200cc. skimmed milk, oatmeal.	3490	3776	3776	Killed be- cause of serious con- dition.
Mar. 11	3	74	147	200cc. skimmed milk, oatmeal.	2170	2586	2586	Killed for examination.
Mar. 11	4	74	147	40 gms. cond. milk.	3255	4100	4100	Killed for examination.

In the later experiments in which equal quantities of skimmed milk and of whole milk were compared, the results were similar to those in the first experiment. S-1, the largest of the four puppies that went on experiment in April, 1921, was the most

severely affected of any of our experimental puppies. She received the skimmed milk ration. After a month on ration she walked with difficulty and would sometimes bark for long periods. She was so sensitive that she barked violently at a sudden noise and became so excited that it was difficult to handle her. At last she refused food and she was killed in order that the effect of the ration might not be masked by the effect of starvation. In contrast to S-1, W-2 was the smallest puppy that we have been able to raise. He received the whole milk ration. He was less active than the others and did not keep himself clean as the others did. He gradually improved while on experiment and at the end could jump higher than any of the others.

S-2 on skimmed milk ration and W-1 on whole milk ration were intermediate in size and in the effect of the ration.

Of the puppies that went on experiment the first of May, 1921, there was less contrast between S-3 on the skimmed milk ration and W-3 on the whole milk ration than there was between the puppies on these rations earlier in the year. The late spring and summer of 1921 were extremely hot, so the greater exposure to sunlight and smaller consumption of oatmeal may account for the lesser difference in effect of the skimmed milk and whole milk rations. At the end of the experiment S-3 was very emaciated but was far livelier and more aggressive than W-3. W-3 on the other hand had a much sleeker coat and was fatter than S-3. At necropsy W-3 was found to have slight broncho-pneumonia and nephritis, while S-3 was in excellent condition except for the extreme emaciation.

#### EFFECT OF HOT WEATHER.

In the very hot summer of 1921, the puppies ate little oatmeal although they continued to be eager for the milk. Those that received skimmed milk became very emaciated. In the winter warm oatmeal was very attractive but in the hot weather they soon tired of it.

One entire litter of six which were received the middle of July, 1921, died within three weeks. There was no apparent difference between those with the whole milk and those with the skimmed. Of a litter of four which were received in June, 1921, three died within a month and the fourth, Dog 10, died of pneumonia after six weeks on the ration of 100 cc. of skimmed milk and oatmeal.

The bones were normal and except for the pneumonia and the emaciation the general condition was excellent.

Attempts were made by the use of meat and of potato to stimulate the appetite but without success. The puppies refused to eat it.

Puppies born in August and in September of 1920 were received in the laboratory stunted and infested with stomach worms. But one of each litter lived. Control Dog B developed into a fine healthy dog. Dog A received the skimmed milk ration. See Table III. Dog A remained stunted and became hump-backed and pot-bellied like Dog 3.

TABLE III.  
EXPERIMENTS WITH WHOLE AND SKIMMED MILK

Date 1920	Dog	Age in Days		Ration	Weight in gms.			Remarks
		Initial	Final		Initial	Max.	Final	
Oct. 14	A	31	83	100cc. skimmed milk, oatmeal, milk increased to 200cc. Nov. 12.	985	1534	1534	Killed for examination.
1921 April 12	S1	40	96	100cc. skimmed milk, oatmeal.	1850	2610	2610	Killed because of serious condition.
" "	S2	40	111	" " "	1200	2300	2300	Killed for examination.
" "	W1	40	111	100cc. whole milk, oatmeal.	1400	2850	2850	" "
" "	W2	38	109	" " "	850	1700	1700	" "
" 30	W3	38	122	" " "	1200	2860	2500	" "
" "	S3	38	122	100cc. skimmed milk, oatmeal.	1620	2510	2420	" "
June 24	10	37	79	" " "	1420	1460	1430	Died of pneumonia. Very emaciated.

## DISCUSSION OF THE RATION.

It is clear that increased consumption of oatmeal would increase the ratio of phosphorus to calcium, which increase Shipley, Park, McCollum and Simmonds<sup>5</sup> have shown to result in rachitic lesions when the ration is deficient in vitamin A. The ash analysis of oatmeal is<sup>4</sup>

Ca	Mg	K	Na	P	Cl	S	Fe
.069	.110	.344	.062	.392	.069	.202	.0038

The proportion of phosphorus to calcium in the oatmeal alone without the corrective effect of the milk would not be high in a ration adequate in other respects. The addition of salt to the oatmeal corrected the sodium-to-potassium balance and increased the chloride. The milk corrected the calcium-to-magnesium balance. The protein of the ration had a fairly high biological value. This value increased with the approach of summer as the milk consumption remained constant while the amount of oatmeal consumed diminished until in the extreme heat of the summer, 1921, it was scarcely touched.

## COMPARISON OF RATIONS.

Due to the seasonal variation in the composition of milk, a ration which contains milk is not constant throughout the experiment or from one experiment to another. Hess, Unger, and Supplee<sup>6</sup> have shown a difference in calcium, phosphorus, sulphur, and citric acid in addition to the difference in vitamin content.

With the changing composition of the milk taken into consideration, the ration, from May to July, is more adequate than the similar ration from April to June. Therefore S-3 received a more adequate ration than S-1 or S-2 and W-3 a more adequate one than W-1 or W-2. Likewise from April to June the ration must have improved so that S-1 on experiment to June 7 was less adequately supplied than S-2 on experiment to June 22. Although of the same liter S-1 was larger than S-2 so in terms of skimmed milk per kilo body weight her ration was more severe throughout.

The duration of the experiment and the ration were the same for W-1 and W-2 but W-1 was the larger puppy so in terms of whole milk per kilo body weight her ration was less adequate.

The first group of experiments was carried out in 1920 and the second in 1921, so the comparison is not as close as it would be

in the same year. The larger amount of skimmed milk received in February may not have contained more vitamin than the smaller amount in June. The consumption of oatmeal was greater in February than in June so that the phosphorus-calcium ratio may have been greater with the larger amount of skimmed milk.

Of the 3 puppies that received skimmed milk in February No. 1 was the largest and No. 3 the smallest. The experiment for No. 1 ended in March and for No. 3 in June, so in all respects the ration of No. 1 was more severe. No. 2 was intermediate between 1 and 3 in initial weight and length of experiment. Even before the vitamin content is increased in the milk the increased exposure to the sunlight must tend to supplement the ration low in "A." We kept no record of cloudy weather but we expect that to be greatest in April.

Taking as many factors as we appreciate at present into consideration S-1 may be considered to have the most extreme experimental conditions and S-2 would come next. S-3, due to difference in season received a much better ration than S-1 or S-2. As skimmed milk contains approximately half as much vitamin A as whole milk, the ration of Dogs 1 to 3 would contain the same amount of vitamin A and a better protein and inorganic content than that of the dogs of the W series if the experiments were carried out at the same time. However, the seasonal differences make the ration of Dogs 1 to 3 more comparable to that of S-3 than to the W series. Table IV gives a comparison of the vitamin A content of the ration for the individual puppies. Of the puppies that received whole milk all were gaining at the end of 10 weeks. W-1 and W-2 were not continued beyond 10 weeks on the experiment. W-2, a stunted puppy, improved throughout the experiment while the others were less lively at the end. W-3 suffered from the intense heat so it was impossible to determine the effect of the ration. No. 4 had the sleekest coat and the most normal appearance of any of the experimental puppies. His weight was stationary after 13 weeks on ration.

#### PATHOLOGY.

Each animal of the series was autopsied and the organs and bones examined both grossly and microscopically. The puppies, fed on the diet deficient in the vitamin A, showed a definite departure from the normal picture. Grossly, pot belly, enlarge-



TABLE IV.  
A COMPARISON OF THE INDIVIDUAL PUPPIES.

Designation of Animal	Initial Weight	Probable Comparative Amount of "A"	Rate of Growth	Length of Experiment in Weeks	Time of Year	Time of Appearance of Effect of Ration Weeks	Bone Lesion
1	High	Medium	High	6	Feb.-March	Mild at 6	Definite
2	Medium	"	"	12	Feb.-April	Severe at 12	More severe than No 1
3	Low	"	"	16	Feb.-June	Marked at 12	Similar to 2
4	High	High	"	16	Feb.-June	Wt. stationary at 13	Nearer normal than 1 to 3
W-1	Medium	Medium	"	10	April-June	Bowlegs at 8	More calcification than in S series. Irregular line
S-1	High	Very Low	Low	8	April-June	Severe at 5	Most severe of all
S-2	Low	Low	Medium	10	April-June	Severe at 8	Similar to S-1 but less severe
W-2	Very Low	High	Low	10	April-June	Improvement on ration	Nearest to normal
S-3	High	Medium	Low	12	May-July	Emaciation hot weather	More calcification than S-2
W-3	Medium	High	Medium	12	May-July	Infection at 9 weeks	Better than W-1

ment of the epiphyses, nodular enlargement of the costochondral junctions were evident. Microscopically, the changes in the costochondral junctions were most obvious. There is an irregularity of the line of ossification varying in degree in the different animals, most marked in Dog S-1. The perichondral and marrow vessels invade the cartilage and in some instances the lamellae of bone seem to contain little calcium and are surrounded by narrow zones of osteoid tissue.

The spleen and lymph nodes in all the experimental animals show some hyperplasia of the Malpighian bodies. In most of the animals the pulp is also more cellular than normal and giant cells are fairly numerous in all sections. The most striking change apart from the bone lesions was observed in the thyroid gland. The acini of the "S" series are larger than those of the "W" series and filled with colloid whereas the thyroids of the "W" series are much more meaty on section and microscopically show less colloid, and are definitely hyperplastic.

Following are more detailed descriptions of some of the sections.

#### Dog A (Normal Control).

This slide from the costochondral junction of a young dog presents a picture of apparently normal ossification. The hyaline cartilage is regular in appearance with the cells uniform. The preparatory striated zone is narrow and has sharply defined straight even margins. It presents the characteristic changes from the fairly regular rows of slightly enlarged cartilage cells next to the hyaline cartilage through the region of much enlarged cartilage cells with calcified ground substance between the rows.

The line of ossification is straight and uniform. Strands of calcified tissue pass from the zone of calcified cartilage into the zone of osseous tissue. The marrow vessels are visible in orderly arrangement all along the line of ossification. They are normal in size and in no instance do they invade the cartilage zone. Beyond this line are the somewhat regular strands of newly formed bone showing calcified cartilage matrix at the center of each trabecula. Between these trabeculae are small spaces filled with marrow cells. Towards the bony end of the section, we find the osseous tissue disposed in larger trabeculae with larger marrow spaces between. Osteoblasts, large giant cell osteoclasts and the various primitive marrow cells are visible. The osteoclasts are numerous.

#### CONTROL DOG 3.

This section and others from this animal present a picture similar to that described for Control Dog A. The animal is older and a larger amount of calcium in the trabeculae is brought out by the ordinary haematoxylin and eosin stain. Osteoclasts are less numerous.



FIG. 1. - Normal Control. Same stock as experimental dog. Same age and weight as Dog 2.

FIG. 2. Dog 2. Received skimmed milk February to April.



FIG. 3 and 4. - Dog 3. Received skimmed milk February to June.







FIG. 5 Dog 4. Received 40 grams condensed milk February to June.



FIG. 6 Dog W1. Received 100 cc. whole milk April to June.



FIG. 7.—Dog W2. Received 100 cc. whole milk April to June.

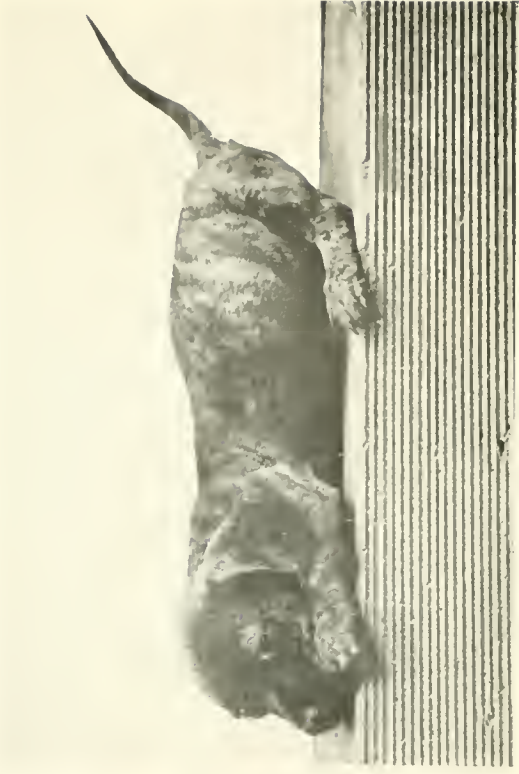


FIG. 8.—Dog S2. Received 100 cc. skimmed milk April to June.







FIG. 9.—Dog S3. Received 100 cc. skimmed milk May to July.



FIG. 10.—Dog A. Received 100 cc. skimmed milk October to December.





FIG. 11—Normal control Costochondral junction Magnification 140 x

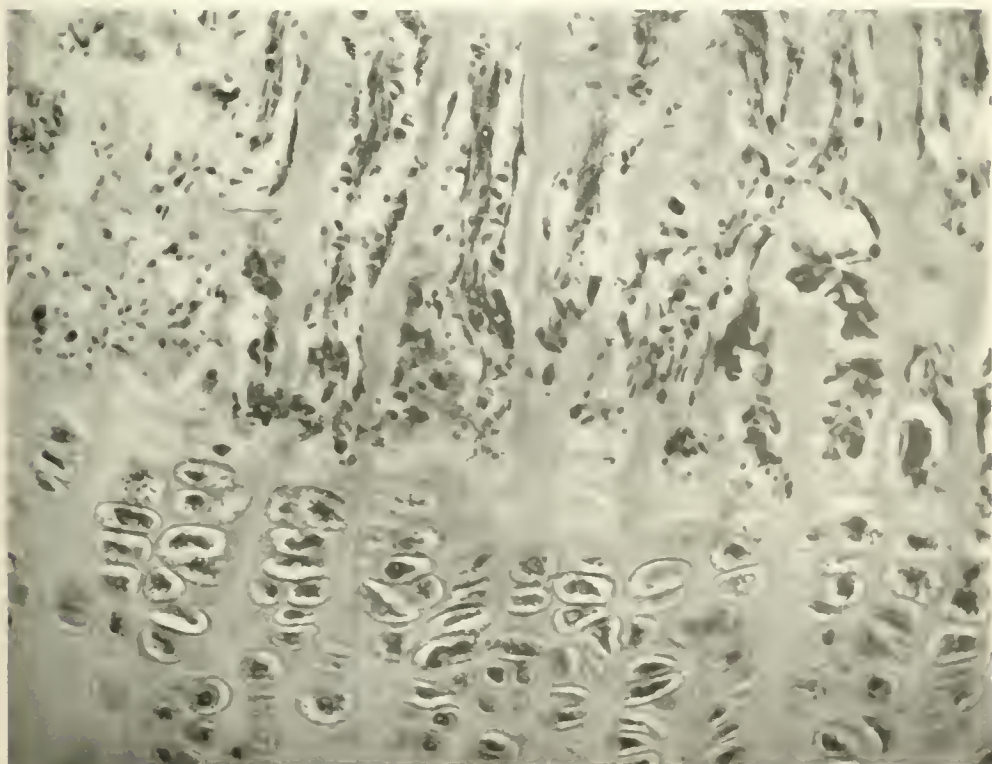


FIG. 12 Normal control Costochondral junction Magnification 265 x





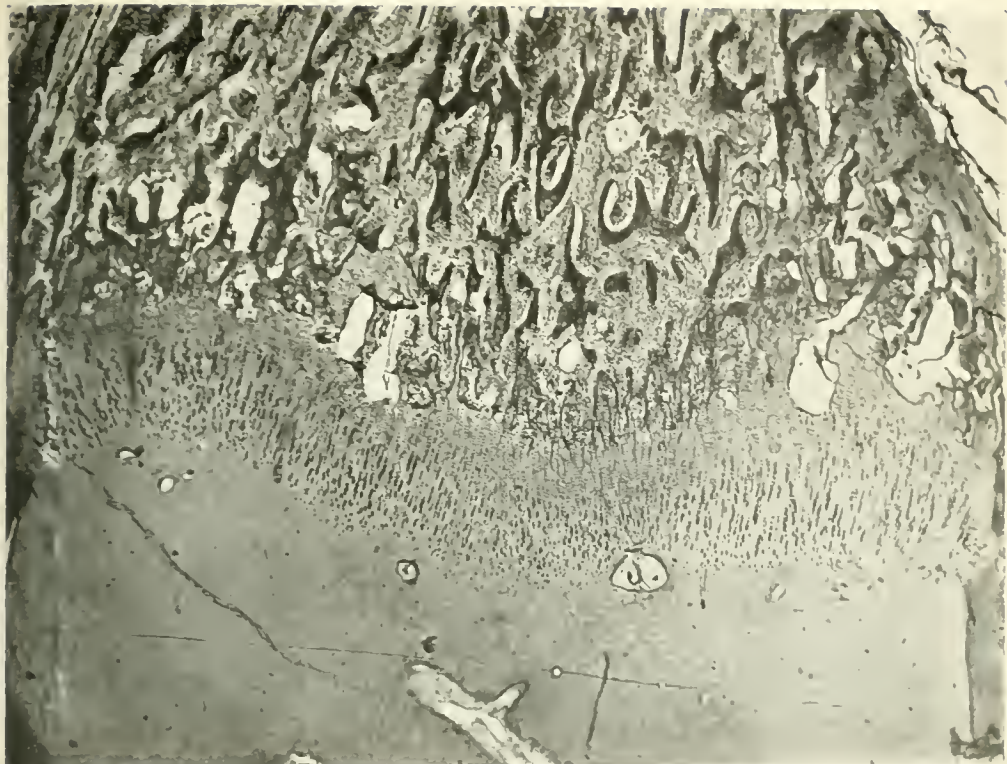


FIG. 13—Dog W2 Costochondral junction Magnification 30 x

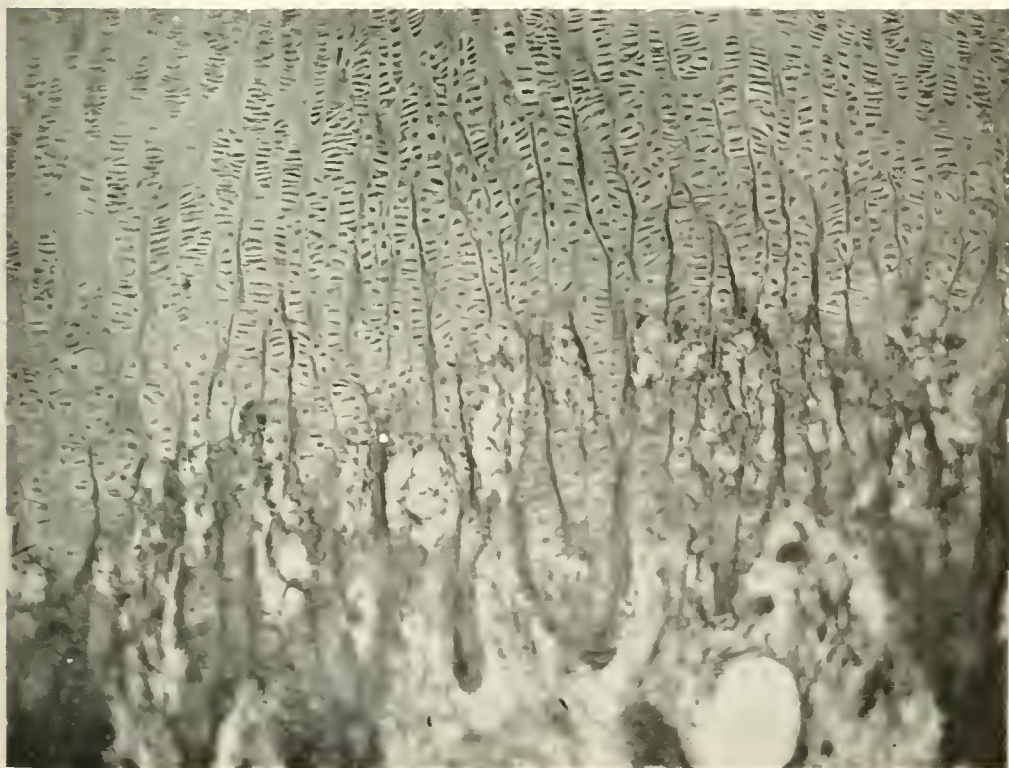


FIG. 14—Dog W2 Costochondral junction Magnification 140 x



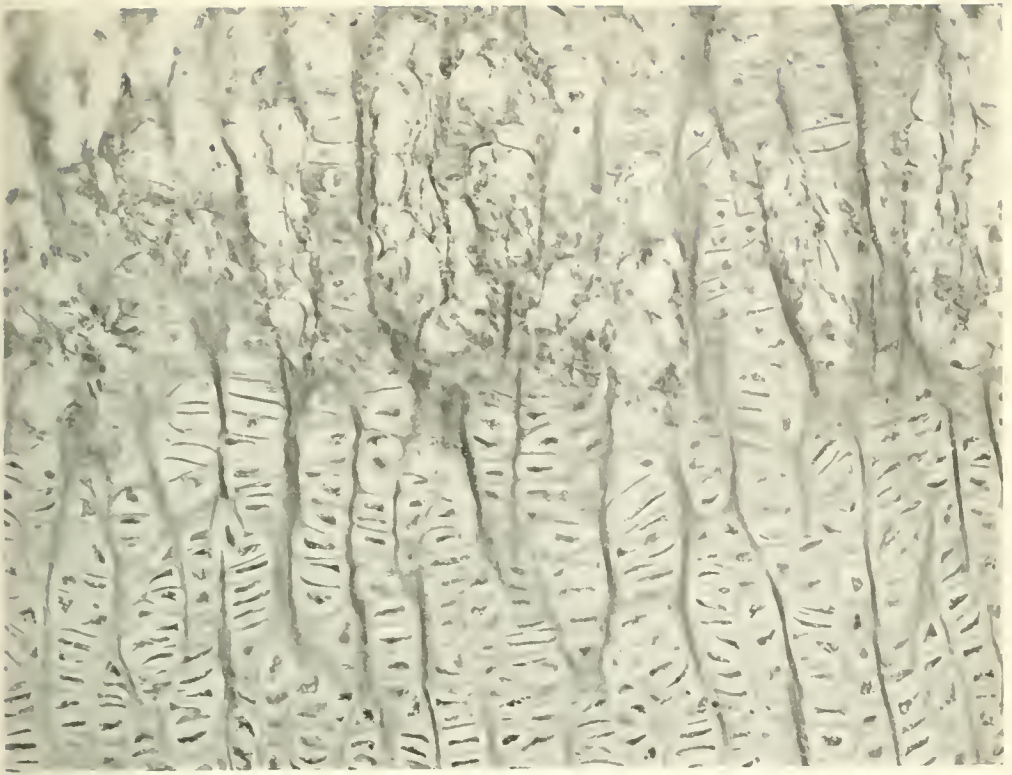


FIG. 15—Dog W2 Costochondral junction Magnification 265 x

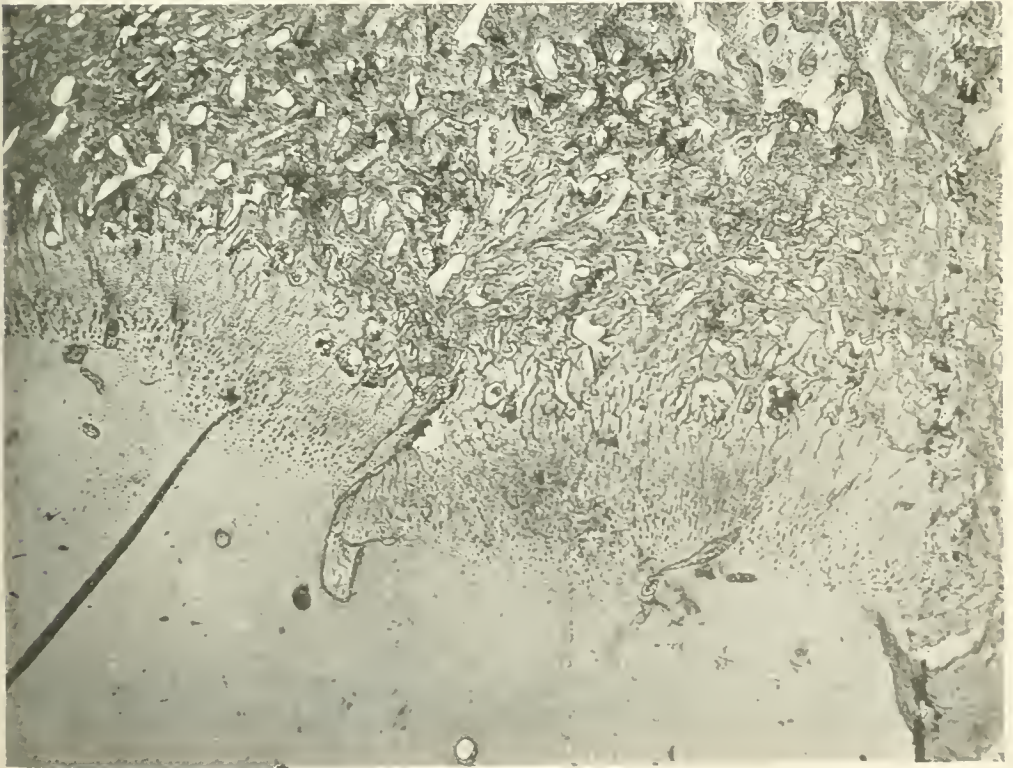


FIG. 16—Dog S2 Costochondral junction Magnification 30 x





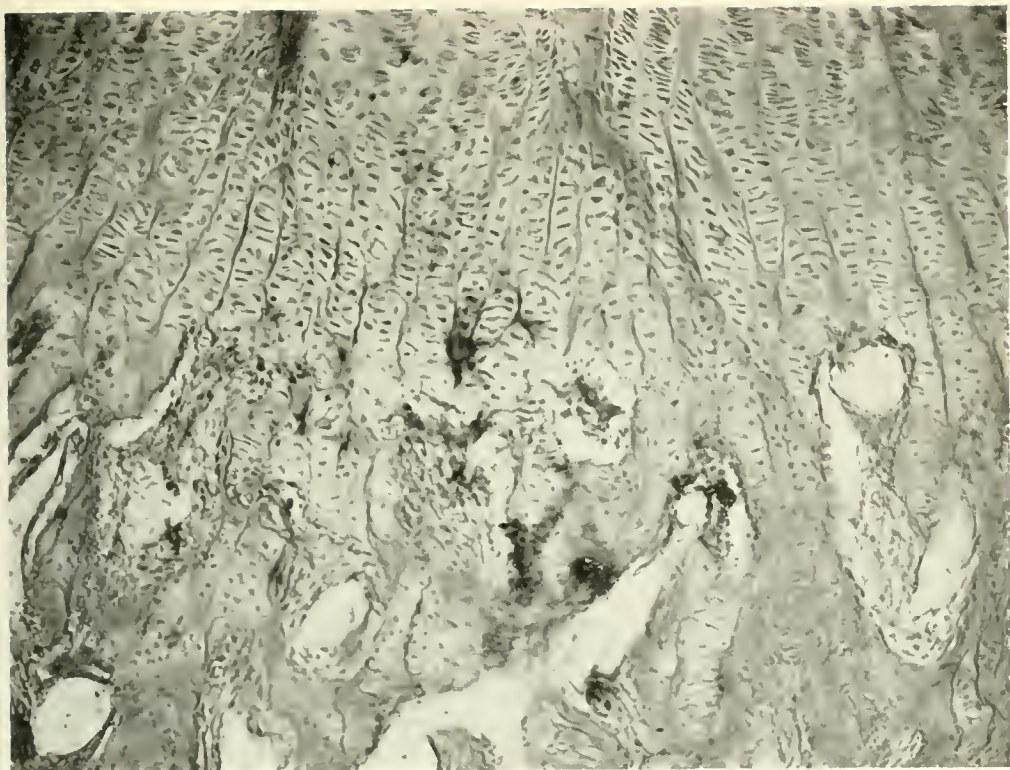


FIG. 17.—Dog S2 Costochondral junction Magnification 140 x

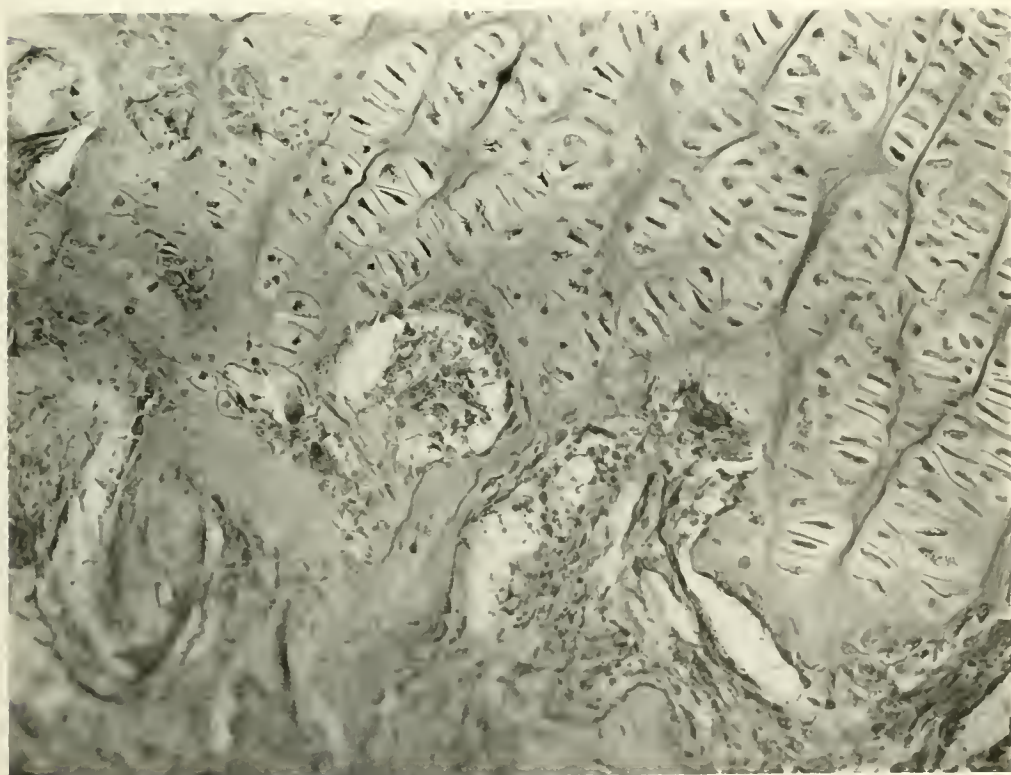


FIG. 18.—Dog S2 Costochondral junction Magnification 265 x





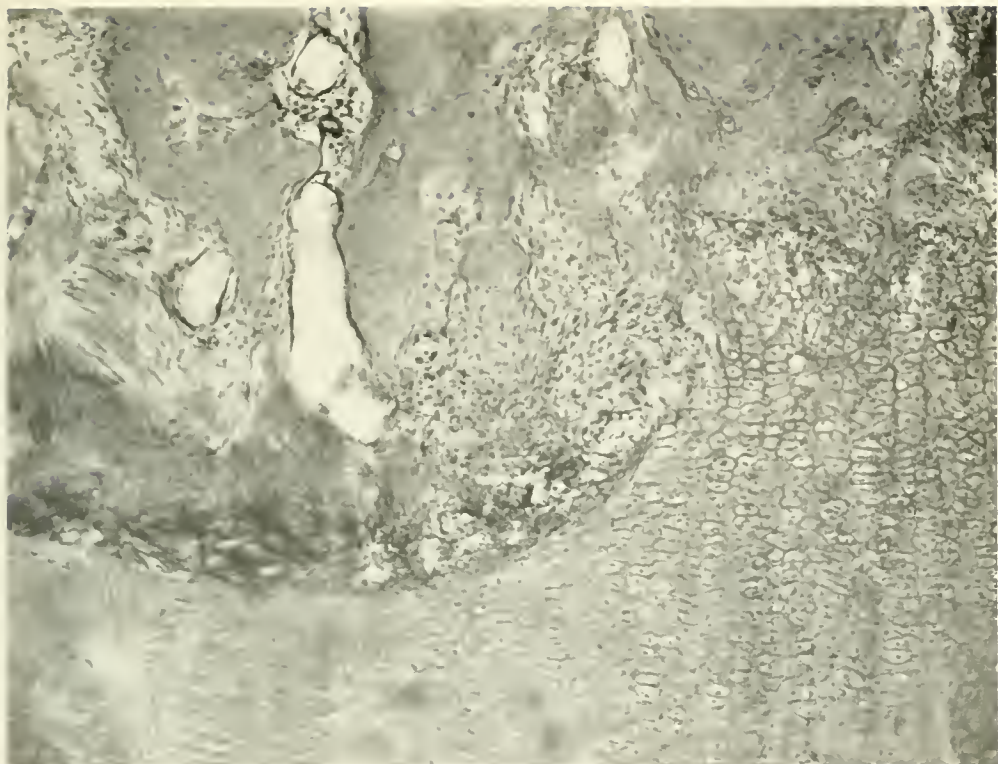


FIG. 19.—Dog S1 Costochondral junction Magnification 140 x

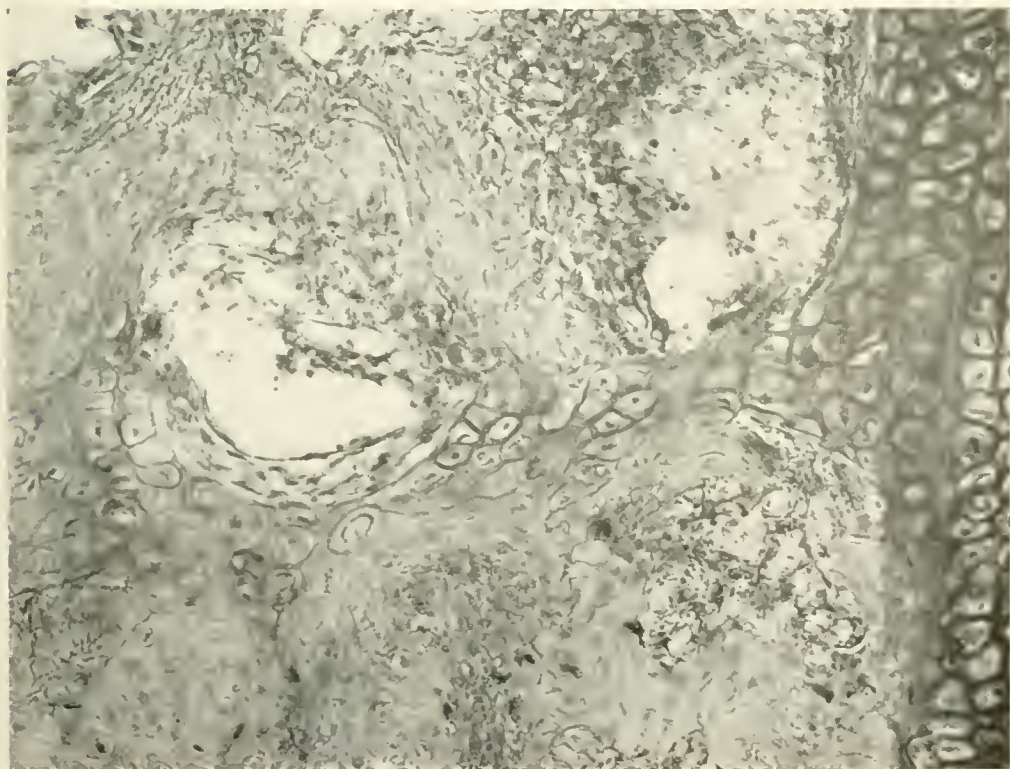


FIG. 20.—Dog S1 Costochondral junction Magnification 265 x





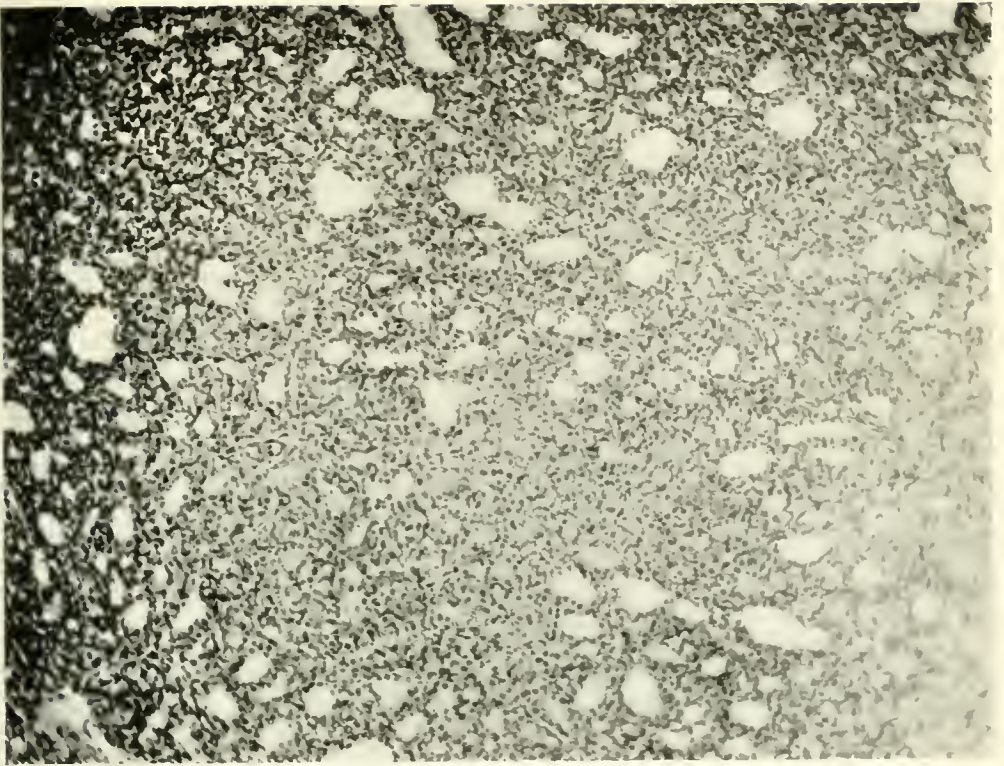


FIG. 21—Dog W2 Thyroid Gland Magnification 140 x

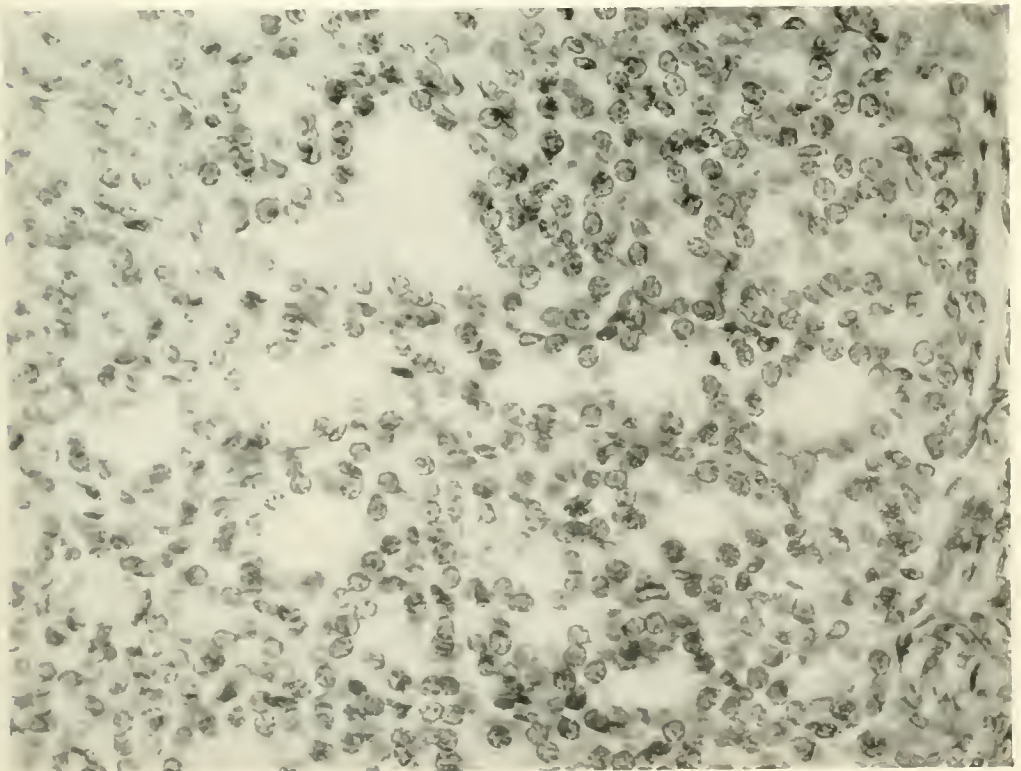


FIG. 22—Dog W2 Thyroid Gland Magnification 550 x





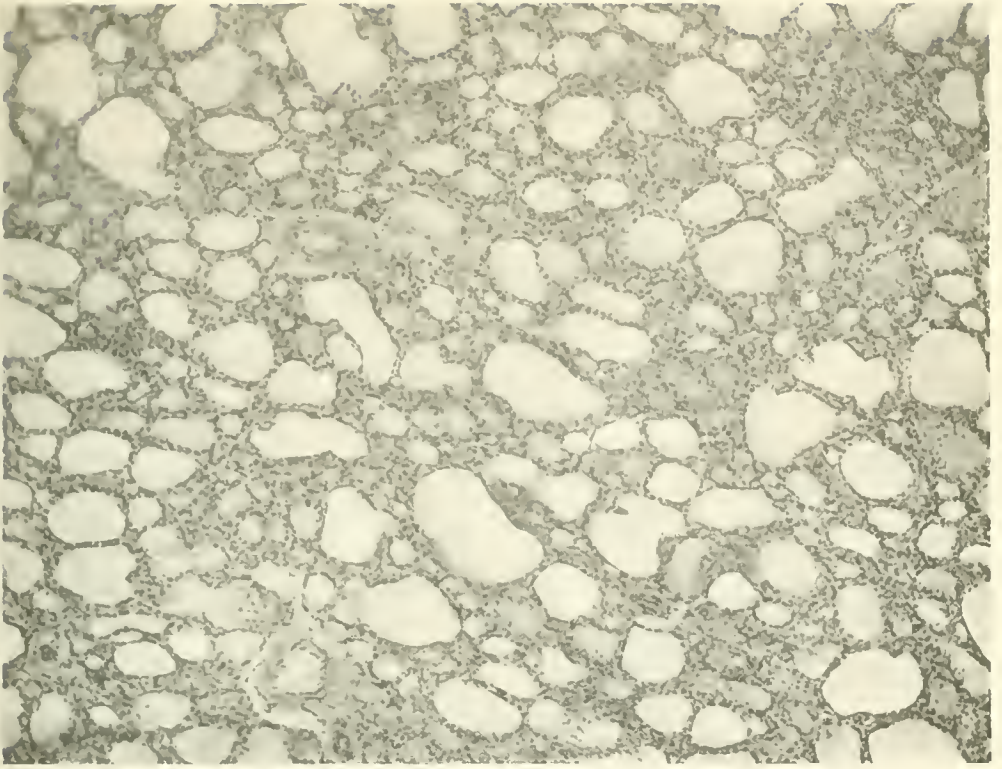


FIG. 23—Dog S2 Thyroid Gland Magnification 140 x

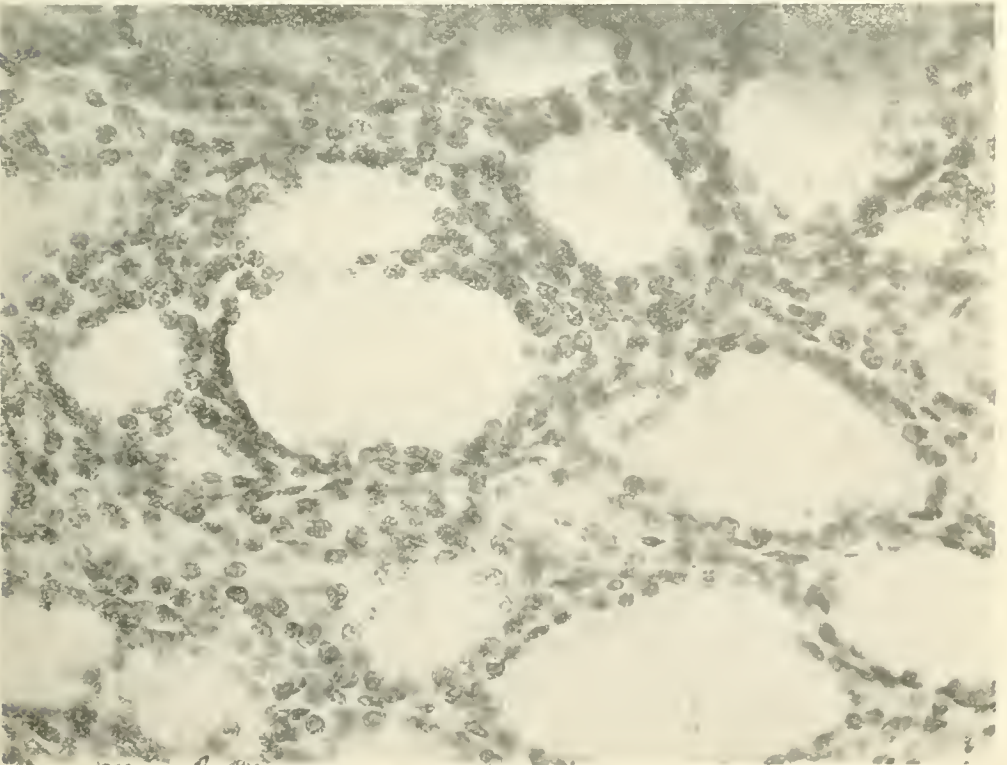


FIG. 24—Dog S2 Thyroid Gland Magnification 550 x



## DOG No. 1.

Sections from the costochondral junctions of this animal show a definite departure from the normal. The striated proliferative zone of cartilage is wider and irregular. Definite invasion of the cartilage by the perichondral and marrow vessels, irregular tongues of cartilage cells projecting down into the bony portions and in general a marked irregularity of the line of ossification present a picture similar to that found in early rickets. The formation of bone is definitely disorganized and there is a thin layer of so-called osteoid tissue surrounding the bony lamellae which are more irregular and apparently contain less calcium than in sections from normal animals of the same age. Osteoclasts are numerous and apparently active.

## DOG No. 2.

The striated proliferative zone of cartilage is less wide than in Dog 1. There is more osteoid tissue and the islands of cartilage cells are found in more remote portions of the shaft. Otherwise the same confused picture is presented as in Dog No. 1.

Dog 3—Similar to Dog No. 2.

Dog 4—Microscopically the divergence from normal is less marked than in 1, 2 or 3. The same elements are present, however, irregularity of the line of ossification, some invasion on the part of the marrow cells, etc.

## DOG S-1.

Sections from the costochondral junctions of this animal show a wider "striated" zone of cartilage than any other animal in the series with even greater irregularity in the line of ossification. A much confused picture is apparent with the marrow and perichondral vessels invading the cartilage vigorously and a considerable amount of osteoid tissue. Slides from the long bones show a similar loss of the normal architecture.

## DOG S-2.

The lesions microscopically are less severe than those in Dog S-1, but there is the same general picture with a confused irregular growth, with invasion of the cartilage by the vascular loops and imperfect and irregular ossification.

## DOG S-3.

Similar to S-2, but less severe. A greater degree of calcification may be noted especially.

## DOG W-1.

Sections show an irregular line of ossification with invasion of the cartilage but there is more perfect calcification than in the slides from the S series. The arrangement of the trabeculae is not normal but there seems to be very little osteoid tissue.

## DOG W-2.

Microscopically the tissues from this animal present a picture nearest to the normal of any in the series. The line of ossification is still somewhat irregular but there is distinctly less invasion on the part of the marrow vessels. Calcium deposits excellent.

## DOG W-3.

The sections show more invasion and greater irregularity in line of ossification than in W-2. There are islands of cartilage cells deeper in the shaft, apparently less calcium deposited and there is some osteoid tissue surrounding the trabeculae.

## SUMMARY.

The degree of departure from normal ossification parallels the degree of departure from normal in a general way. There is, however, less distinction between the whole milk and skimmed milk series in the bone lesions than there was in general condition.

The reason that bone lesions are prominent on inadequate rations is doubtless due to the fact that bones of young animals are rapidly differentiating tissue. The interesting investigation of Cramer, Drew and Mettran<sup>7</sup> on blood platelets in vitamin A deficiency, the observations of Mellanby<sup>8</sup> and ourselves on the thyroid and the changes in the spleen suggest that there is a wide spread change in metabolism that has not as yet been traced to its source.

The similarity found by Tozer<sup>9</sup> in the bones of guinea pigs deprived of A and of C vitamins respectively is further evidence that the bone lesion is a secondary result of the ration.

The condition known as rickets is possibly diagnosed from the secondary symptoms of dietary deficiency. This would account for the diverse causes assigned to it.

## CONCLUSIONS.

1. Young puppies fed a ration containing sufficient vitamin A for growth but not enough for prolonged maintenance develop bone lesions characteristic of early rickets.
2. The severity of the lesions is in inverse proportion to the vitamin A content of the ration.
3. The difference in general condition is much greater than the difference in bone lesions on the different levels of vitamin A.
4. It is probable that the bone lesion is a secondary effect of a change in metabolism due to insufficient vitamin A.
5. Aside from the bone lesions, the most striking difference between the puppies that received whole milk and those that received skimmed milk was found in the thyroid.

## REFERENCES.

1. Powers, Park, Shipley, McCollum, and Simmonds. *J. Amer. Med. Assn.*, 78, 1922, 159.
2. Hess, Unger, and Pappenheimer. *J. Biol. Chem.*, 50, 1922, 77.
3. Mellanby. *Med. Res. Council Special Report Series*, No. 61, 1921.
4. Quoted by Sherman. *Chemistry of Foods and Nutrition*, MacMillan Company, 1918.
5. Shipley, Park, McCollum, and Simmonds. *Amer. J. Dis. Chil.*, 23, 1922, 91.
6. Hess, Unger, and Supplee. *J. Biol. Chem.*, 45, 1920, 229.
7. Cramer, Drew, and Mottram. *Proc. Roy. Soc.*, 93B, 1922, 655.
8. Mellanby. *Proc. Physiol. Soc., J. of Physiol.*, 55, 1921, VII.
9. Tozer. *J. Path. and Bacteriol.*, 24, 1921, 306.





# EFFECT OF VARIOUS RATIONS ON YOUNG NORMAL GUINEA PIGS AND ON YOUNG GUINEA PIGS INOCULATED WITH TUBERCULOSIS.

MARGUERITE DAVIS.

*From the Home Economics Laboratory, University of Wisconsin.*

As it is accepted that fat and particularly a fat with a high biological value such as milk fat is beneficial in the treatment of tuberculosis, a series of experiments was planned to study this point.

Young guinea pigs and half grown rabbits were inoculated after a preliminary feeding period and continued on their respective rations.

One ration consisted of crushed oats, salt, boiled potato, and unpasteurized skimmed milk received fresh each morning from the University Dairy barn. We have found this to be an inadequate ration for rats.<sup>1</sup>

In the second ration pasteurized whole milk received each morning from a local dealer replaced the skimmed milk of the first ration. This ration has proved adequate for rats.

A third lot received crushed oats, salt, hay, cabbage, carrot, and tap water, a ration which we have used for our guinea pig breeding stock and refer to as "stock ration." For the fifty days before inoculation, growth was better on the "stock ration" than on either of the milk rations for both guinea pigs and rabbits. With the rabbits there was more increase in weight on the whole milk ration than on the skimmed milk ration. With the guinea pigs the food consumption was poor on each of the milk rations and they became emaciated.

The inoculation was with a bovine culture virulent for guinea pigs but which proved non-virulent for the rabbits.

The weight curves of the rabbits that received the skimmed milk ration were less affected by the inoculation than the curves of the other two lots. After seven months on ration and two months after a reinoculation with the same strain of bovine tuberculosis, the rabbits were killed and examined.

Those that received the whole milk ration had glossier coats than the others but otherwise the animals of the three lots could not be distinguished from one another either externally or at necropsy. All showed evidence of tuberculosis.

In spite of the superior condition of the guinea pigs that received the "stock ration," they showed less resistance to tuberculosis than those that received the two milk rations. Some individuals on the skimmed

milk ration seemed particularly resistant. In order to determine whether this was accidental or due to the ration, another series of experiments was carried out.

The second series extended from the first of July into October, a season when both milk and potatoes have greater food value than in the winter and early spring, which was the time of the preliminary experiment. Also the animals drink more in hot weather, and as their total consumption of food is less, the milk forms a greater proportion of the ration.

The purpose of this series was to investigate the effect of milk during the pre-inoculation and post-inoculation periods as well as during the entire experiment. Young guinea pigs were divided into seven lots. Three groups received their respective rations throughout the experiment. For the other four groups, the rations were changed at the time of inoculation. Inoculation was made with a culture from a guinea pig that received the "stock ration" during the preliminary experiment and took place after one month of preliminary feeding.

#### RATION CONTINUOUS THROUGHOUT EXPERIMENT.

Lot 1 received crushed oats, salt, hay, cabbage, carrot and tap water, called "stock ration."

Lot 2 received the same ration as Lot 1 except that the tap water was replaced by skimmed milk.

Lot 3 received crushed oats, salt, boiled potato, and skimmed milk, called "skimmed milk ration."

In the pre-inoculation period the rate of growth was greatest for Lot 2 and least for Lot 1.

Lot 2 fared better than Lot 1, see Table 1.

Lot 3 had the poorest group record and the best individual record. The individual record would seem an accident if it were not a repetition of what was found in the preliminary series, and for the difference found at necropsy between Lot 3 and Lots 1 and 2.

#### RATION CHANGED AT TIME OF INOCULATION.

Lots 4 and 5 received the "stock ration" up to inoculation.

Lot 4 was changed to crushed oats, salt, boiled potato and whole milk, called "whole milk ration."

Lot 5 was changed to the "skimmed milk ration," in which skimmed milk replaces the whole milk of "whole milk ration."

The record of Lot 4 after inoculation was surprisingly uniform. They lost weight rapidly and died from three weeks to a month after inoculation.

Lot 5 remained stationary in weight and survived longer than Lot 4, see Table 1.

It is hard to estimate degree of infection with tuberculosis, as different organs are affected to different degrees. There was, however, a striking difference in the condition of one of Lot 5 and two of Lot 1 that died two months after inoculation. In Lot 1 there was an advanced stage of generalized tuberculosis, in Lot 5 there was a definite but not severe tubercu-

losis and death may have been due to pneumonia. Lot 5, on the other hand, gave evidence of greater susceptibility to tuberculosis than Lot 3.

Lot 6 received the "skimmed milk" ration up to inoculation and then the "stock ration." The record of this lot is by far the best of the seven. This may be largely due to the increased consumption of food due to the change to a more palatable ration.

Lot 7 received the same ration as Lot 2, the "stock ration" with tap water replaced by skimmed milk, in the pre-inoculation period, and the plain "stock ration" with the tap water after inoculation.

The record of Lot 7 closely resembles that of Lot 1. The adverse effect of a less adequate ration after inoculation may offset the beneficial effect of the milk in the pre-inoculation period. The consumption of milk is not as great when there are succulent vegetables in the diet as it is on the "skimmed milk ration," therefore Lot 7 cannot be compared with Lot 6 in the amount of milk consumed in the pre-inoculation period.

TABLE 1.  
PERIOD OF SURVIVAL ON DIFFERENT RATIONS

Lot	Pre-Inoculation Ration	Post-Inoculation Ration	No. of Animals	Days of Survival After Inoculation	Number Surviving at 65 Days
1	"Stock"	"Stock"	6	36, 49, 56, 60, 60	1
2	"Stock" with Skimmed Milk	"Stock" with Skimmed Milk	9	27, 43, 49, 54, 62	4
3	"Skimmed Milk"	"Skimmed Milk"	12	21, 21, 25, 29, 30, 33, 34, 39, 44, 52	2*
4	"Stock"	"Whole Milk"	8	20, 21, 24, 27, 28, 29, 30, 31	0
5	"Stock"	"Skimmed Milk"	13	23, 26, 28, 32, 32, 34, 35, 37, 38, 39, 40, 40, 61	0
6	"Skimmed Milk"	"Stock"	11	43, 45, 48, 58	7
7	"Stock" with Skimmed Milk	"Stock"	7	43, 45, 46, 46	3*

\*1 of Lot 2 and 2 of Lot 7 were half grown animals.

#### DISCUSSION OF SECOND SERIES.

A consideration of the result of these experiments brings out the need for quantitative determinations of the effect of various types of food.

If we take the "stock ration" as a standard we find that the substitution of skimmed milk for tap water increases the resistance to tuberculosis. If we compare it with the "skimmed milk

ration" in which the milk is supplied ad libitum but the ration is less adequate in other respects for normal guinea pigs, we find one individual with the best record of any of the 65 guinea pigs and we find in general less evidence of tuberculosis and more congestion of the lungs and pneumonia. The great individual difference on the "skimmed milk ration" is doubtless due to individual differences in milk consumption.

The animals were kept in large pens in an airy, sunny room. There was opportunity for exercise and the food was obtained from a common dish which usually increases the food consumption. Still the food consumption was unsatisfactory on the "skimmed milk" and "whole milk" rations. The better condition of the group that received the "skimmed milk ration" in the pre-inoculation period over the two groups that received skimmed milk with the "stock ration" is striking. Here again the amount of milk consumed doubtless plays a part and the effect on food consumption of a change from a less adequate to a more adequate diet.

Finally there is the comparison of the "whole milk ration" with the "skimmed milk ration." The condition of the group that received the "whole milk ration" was so definitely the worse that it seemed advisable to investigate this point with normal guinea pigs. Therefore, a third series of experiments was carried out.

### THIRD SERIES OF EXPERIMENTS.

The purpose of this series was to compare the effect of whole and skimmed milk in the diet of the young guinea pig. The whole milk was pasteurized and was supplied by a local dealer. The skimmed milk was also pasteurized and was received from the University dairy and was not the unpasteurized skimmed milk from the University dairy barn used in the earlier experiments.

Sixteen young guinea pigs were placed in individual cages. All received crushed oats, salt and boiled potato. In addition one group received 40 cc. of skimmed milk, another 80 cc. of skimmed milk, another 40 cc. of whole milk, and the fourth 80 cc. of whole milk. The experiment ran from May to September.

As the facilities for individual feeding were limited, groups were fed in pens to serve as controls. One group received the "skimmed milk ration," which differed from the 40 cc. and 80 cc. skimmed milk diet only in that the skimmed milk was supplied ad libitum. A second group received the "whole milk ration," which differed from the "skimmed milk ration" only in the inclusion of the milk fat. A third group received the "stock ration,"



which consisted of crushed oats, salt, hay, cabbage, carrot and tap water. A fourth group received the "stock ration," in which the tap water was replaced by whole milk. These groups were on ration from March to September.

For the first month the lot that received 40 cc. whole milk grew more rapidly than any other group except the one that received 80 cc. whole milk. During the second month all of that lot died after a rapid loss in weight. The lot that received 80 cc. whole milk were still vigorous and growing at the end of the experiment. See Table 2.

The lot that received 40 cc. skimmed milk grew slowly. Three died in the third month. One survived to the end of the experiment, when it was slowly gaining in weight.

Of the lot that received 80 cc. of skimmed milk, one died in the third month after a rapid gain in weight. Two grew at about the same rate as the survivor of the 40 cc. group and were still gaining at the end of the experiment. The fourth was half grown at the start and grew extremely rapidly.

As the animals died others were put in their cages and on their ration, with the same result as was observed in the original group.

Of the lots that received milk ad libitum, those that received skimmed milk consumed much more than those with the whole milk. That probably accounts for the fact that six of eleven survived on skimmed milk while only three of ten survived on the whole milk. Of the six, one grew very rapidly and all were gaining at the end of the experiment. The three that received whole milk gained in weight rapidly for four months, and then lost rapidly so that their weight curves resemble to a striking degree those of the group that received 40 cc. of whole milk.

The difficulty with the "whole milk ration" for the young guinea pig appears to be that if the consumption of milk does not reach a minimum, which is probably near 80 cc., growth is stimulated to too great an extent for the support of normal metabolism. For equal quantities there is in general less rapid growth with skimmed milk in the ration than with whole milk. This difference might disappear or be reversed at high levels of milk consumption.

The addition of whole milk to the "stock" ration increased the rate of growth and improved the general condition of the animal. There was no determination of the milk consumption, but it probably averaged about 40 cc.

## DISCUSSION.

It should be borne in mind that these are experiments with young animals. The guinea pig is unique in that it is independent of its mother from birth. We did not know the age of these guinea pigs as we selected the most vigorous appearing individuals from a group of several hundred. The average weight for age for guinea pigs is roughly 100 gms. at birth and 400 gms. at 40 days.

In Tables 3 to 6 the animals are divided into weight groups. Not all of the individuals fall within these groups but the groups are representative of the whole number.

These Tables show (1) better growth and greater resistance to tuberculosis with the addition of milk to the "stock ration," (2) extreme lack of uniformity in maintenance, growth and resistance to tuberculosis with the "skimmed milk ration" due without doubt to individual difference in milk consumption, (3) increased resistance to tuberculosis when the "skimmed milk ration" is changed to "stock ration" at the time of inoculation which may be due to the increased food consumption after change in ration and also possibly to the beneficial effect of the skimmed milk in the pre-inoculation period, and (4) unfavorable results with the "whole milk" ration due to the unsatisfactory milk consumption.

Table 2 shows that 80 cc. of whole milk is a more adequate addition to oats, salt and potato than 80 cc. of skimmed milk, and that 40 cc. of skimmed milk is a more adequate addition than 40 cc. of whole milk. The superiority of the smaller amount of skimmed milk is apparently due to the slower rate of growth if increase in weight may be taken as rate of growth. This is in accord with the observations of Mellanby<sup>2</sup> which have been confirmed in this laboratory that the pathological changes in puppies growing rapidly on an inadequate ration are more severe than in slow growing puppies.

#### NATURE OF THE DEFICIENCY.

Guinea pigs that died on the "whole milk" and "skimmed milk" rations presented a strikingly uniform appearance at necropsy. The most prominent features were enlargement of the mesenteric lymph nodes and pigmentation from blood destruction. The Peyer's patches were deeply pigmented. Fatty degeneration of the liver was frequently found. The spleen was not greatly affected but frequently showed a slight hyperplasia of the lymph substance. Fatty degeneration of the kidney was occasionally found.

McCarrison<sup>3</sup> reports enlargement and discoloration of the mesenteric glands in monkeys as an effect of an autoclaved rice diet. This diet was very extreme while the rations that we are considering were deficient, so far as we know, only in vitamin A.

When the guinea pigs that received 80 cc. whole milk were killed and examined they presented the same appearance as those that received the "stock" ration. One that had died on the ration appeared normal except for the kidneys. The experiment lasted only four months but it included the major part of the growth period so it seems fair to consider the ration adequate for the growing guinea pig.

Of those that received 80 cc. of skimmed milk three resembled the controls, except that they were not as fat. Two others refused food in the latter part of the experiment, died, and presented the pathological condition described above.

All that received 40 cc. of whole milk and all but one that received 40 cc. of skimmed milk resembled those that died on the "whole milk" and "skimmed milk" ration.

### CONCLUSIONS.

1. Addition of 40 cc. of whole milk to an inadequate ration causes rapid growth followed by rapid loss in weight and death.

2. Addition of 40 cc. of skimmed milk results in slower growth, less loss in weight and longer survival.

3. Animals dying on either ration present at necropsy enlarged mesenteric glands, pigmentation from blood destruction and in some cases fatty degeneration of the liver.

4. The addition of 80 cc. of whole milk results in normal growth and development.

5. With addition of 80 cc. of skimmed milk growth is slower than with the whole milk and the condition is sometimes normal and sometimes resembles that of the 40 cc. groups.

6. Addition of skimmed milk to an adequate ration increases the resistance to tuberculosis.

7. Differences in food consumption on different rations make comparison difficult but it seems probable that skimmed milk has a peculiar beneficial effect in tuberculosis.

8. Experiments with whole milk with inoculated animals were unsatisfactory because of poor consumption of food.

In conclusion I wish to express my appreciation of the assistance of Meta Schroeder of the Department of Agricultural Bacteriology in inoculating the animals and in the examinations.

TABLE 2.  
THIRD SERIES—EXPERIMENT WITH DEFINITE  
AMOUNTS OF MILK.

Milk in Ration	Weight in Grams			No. Days on Ration	Condition
	Initial	Maximum	Final		
400 cc. Skimmed	230	315	250	65	Typical Pathological
	260	340	245	61	" "
	260	500	450	70	" "
	275	560	560	120	Normal
80 cc. Skimmed	250	510	510	120	Normal
	280	620	620	120	"
	290	550	470	70	Typical Pathological
	450	855	855	68	Normal
40 cc. Whole	280	335	260	56	Typical Pathological
	280	420	280	56	" "
	290	425	290	56	" "
	380	430	270	32	" "
80 cc. Whole	245	740	740	120	Normal
	250	490	345	41	"
	270	790	790	120	"
	420	720	720	68	"

TABLE 3.  
SECOND SERIES—GUINEA PIGS WITH INITIAL WEIGHT  
OF 140-200 gms.

Lot	Weight in Grams			Days Survival After Inoculation
	At Time of Inoculation	Maximum	Final	
1	225	330	240	60
	245	325	320	36
	280	310	285	49
	305	360	330	*
2	300	400	375	*
	320	340	315	62
	350	365	360	49
	250	280	250	*
5	240	240	175	23
	240	245	230	32
	245	245	200	39
	250	275	270	34
	260	260	230	32
	270	290	250	61
	275	325	320	40
	285	285	270	28
6	230	375	375	*
	210	245	225	48
7	390	405	395	45
	370	420	370	46

\*Survived to end of experiment.

To Dr. Clark of the Department of Pathology and to Dr. Frost of the Department of Agricultural Bacteriology. I am indebted for advice and assistance throughout.

TABLE 4.  
SECOND SERIES—GUINEA PIGS WITH AN INITIAL  
WEIGHT OF 240-300 gms.

Lot	Weight in Grams			Days Survival
	At Time of Inoculation	Maximum	Final	
1 Males	290 330	300 375	280 300	56 60
2 Females	430 380	490 500	470 360	43 •
3  Males	370 420 380 400	410 490 380 610	340 330 380 610	44 <sup>1</sup> 39 <sup>1</sup> 21 <sup>2</sup> •
4  Females	370 400 440	370 400 440	245 310 300	29 21 31
5 Males	300 380	305 380	295 360	35 40
6 Males	410 440	430 500	405 475	• •
7 Females	420 430	420 490	340 475	46 •

\*Survived to the end of experiment.

<sup>1</sup>Lungs most involved.

<sup>2</sup>T. B. doubtful. Condition similar to that of uninoculated guinea pigs that died on ration.



TABLE 5.  
THIRD SERIES—GUINEA PIGS WITH INITIAL WEIGHT  
LESS THAN 200 gms.

Ration	Weight in Grams			No. Days on Ration	Condition
	Initial	Maximum	Final		
"Stock" Female	150	700	700	180	.....
"Stock" and Whole Milk	124	850	850	180	.....
"Whole Milk" Males	157	200	155	26	Typical Pathological
"Skimmed Milk" Males	140	430	410	150	Typical Pathological
	158	665	665	180	Normal
	190	255	240	26	Typical Pathological

TABLE 6.  
THIRD SERIES—GUINEA PIGS WITH INITIAL WEIGHT  
FROM 200-285 gms.

Ration	Weight in Grams			No. Days on Ration	Condition
	Initial	Maximum	Final		
Stock Females	215	.....	760	180	.....
	235	.....	730	180	.....
	240	.....	800	180	.....
"Stock" with whole milk Females	210	.....	770	180	.....
	225	.....	820	180	.....
	245	.....	800	180	.....
	255	.....	800	180	.....
	265	.....	960	180	.....
"Whole Milk" Males	215	280	230	36	Typical Pathological
	225	725	610	180	Normal
	235	300	270	74	Typical Pathological
	245	245	245	21	" "
	265	305	235	36	" "
	275	320	250	26	" "
"Skimmed Milk" Males	200	240	220	36	Typical Pathological
	225	540	540	180	Normal
	225	315	215	36	Typical Pathological
	225	285	285	27	" "
	240	670	670	180	Normal
	255	660	660	180	"
	260	785	785	180	"
	285	660	660	180	"

## REFERENCES

1. Davis and Outhouse. *Am. J. Dis. Child.*, 21, 1921, 307.
2. Mellanby. *Med. Res. Council, Special Report Series*, No. 61, 1921.
3. McCarrison. *Studies in Deficiency Disease*, Oxford Medical Publications, 1921.



## THE EFFECT OF X-RAY EXPOSURE ON METABOLISM.

BY DR. A. L. B. BARRETO.

*School of Hygiene and Public Health, Johns Hopkins University.*

The very marked changes that occur in the body after the absorption of radiant energy (light, X-rays, radium rays) lead one to suppose that these effects may be accompanied by changes in metabolism. Previous work has shown an increased rate of  $\text{CO}_2$  production in visible light as compared with the dark,<sup>1</sup> an increase in calcium and phosphorus metabolism after exposure to ultra-violet light,<sup>2</sup> an increased nitrogen output after exposure to X-rays,<sup>3</sup> and an increased nitrogen output and rate of  $\text{CO}_2$  production after exposure to radium.<sup>4</sup> There have been, however, no experiments giving the effect of X-ray exposure on the resting metabolism of normal animals. All the published data are concerned with the effect of X-rays on the metabolism of patients with either cancer or leukemia.

Experiments were therefore undertaken to investigate the rate of oxygen consumption in birds after exposure to X-rays. Birds were chosen, partly on account of their high metabolism, but largely on account of the fact that they remain very quiet during the experiments. Mice are so active that it is extremely difficult to get good results with them. Although only six birds were radiated the results obtained were very consistent.

### *Method.*

The experiments consisted in measuring the rate of oxygen consumption in canary birds before and after exposure to X-rays. Meyer's apparatus, which he describes in detail in a recent paper,<sup>5</sup> was used. This apparatus consists of a closed system in which there is a small animal chamber, two flasks partly filled with 1% potassium hydroxide, and two 50 cc. burettes arranged in manometer fashion and connected at the bottom by rubber tubing. These burettes contain glycerinated distilled water. The bird is put in the metabolism chamber and air pumped through, with A and B open and C and D closed, during the preliminary period while temperature and barometric readings are made. The pump is then stopped, A and B are closed, C and D opened, and the oxygen consumption measured for a five minute period. During this period air is circulated through the system by raising and lowering the flasks alternately. At the end of the experiment the burettes are levelled and the oxygen consumption in cc. is gotten directly from the burette readings. This value is

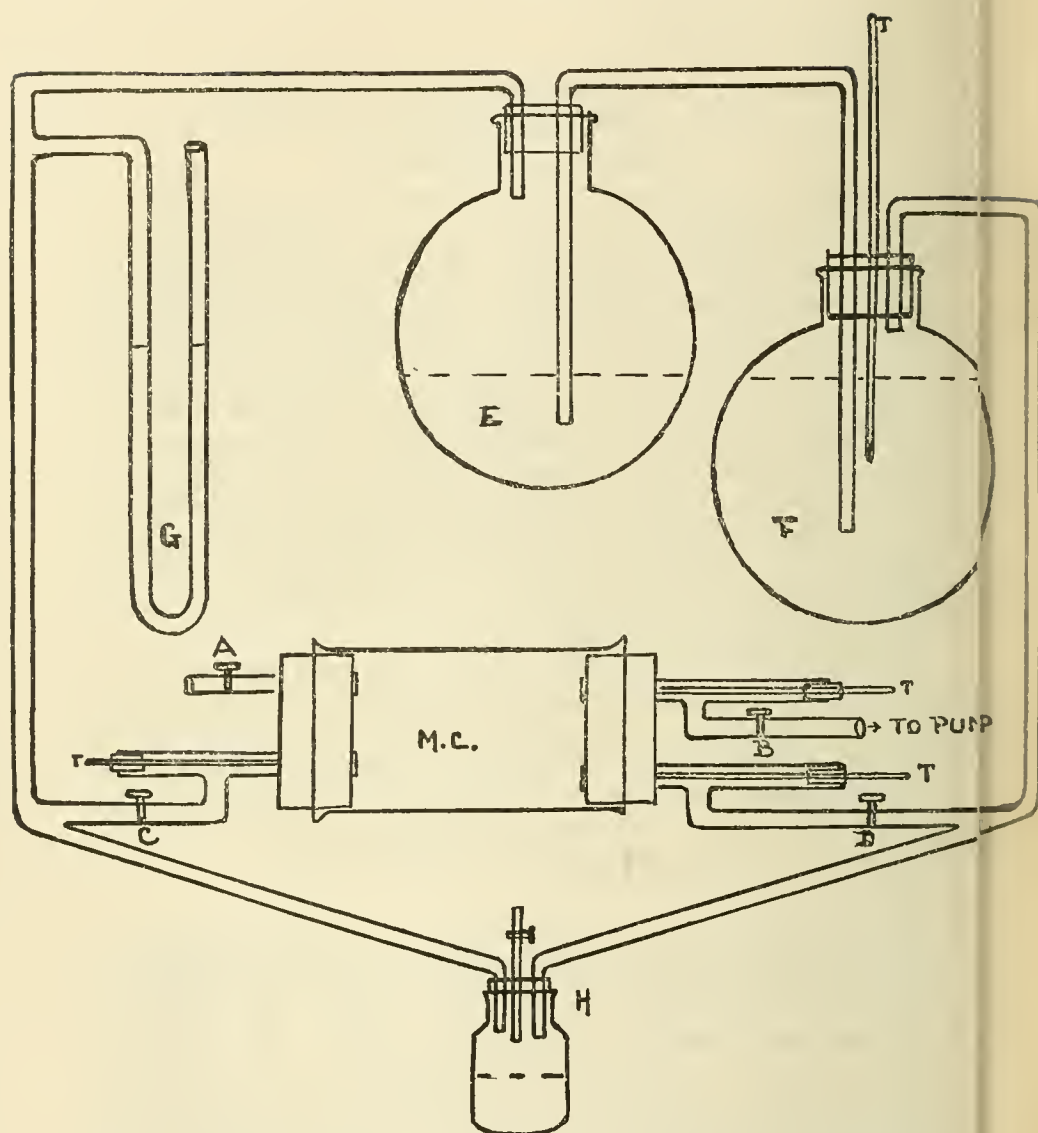


Fig. I.—Diagram of Meyer's apparatus for determining the metabolism of small animals.

T.=Thermometers.

A. B. C. D.=Stopcocks.

M. C.=Metabolism chamber (3 x 2 inches).

E. F.=Flasks containing 1% KOH (capacity of each flask=2200 cc.

G.=Two 50 cc. burettes.

H.=By-pass.



corrected for barometric changes, and for temperature changes in the flasks and animal chamber during the experiment, and is finally reduced to grams of oxygen per kilogram of bird per hour, at normal temperature and pressure. The weight of the bird, including feathers, was used in calculating the results. Fig. 1 shows a diagram of the apparatus and for further details concerning the use of the apparatus the reader is referred to Dr. Meyer's paper.<sup>5</sup>

The birds were exposed, while in the metabolism chamber, to different doses of X-rays from a Coolidge tube, operating at 55,000 volts, which corresponds roughly to a  $3\frac{1}{2}$  inch spark gap. The distance from the anticathode to the bird was  $9\frac{1}{2}$  inches. In calculating the X-ray dose Remer and Witherbee's formula for unfiltered radiation was used:

$$\frac{\text{Spark gap} \times \text{milliamperes} \times \text{minutes}}{(\text{distance})^2} = \frac{p}{r_0} = 1 \text{ skin dose}$$

It is evident that an exposure of about fifteen milliamperere-minutes at a distance of  $9\frac{1}{2}$  inches with a  $3\frac{1}{2}$  inch spark gap will be equivalent to one skin dose. As the bird was enclosed in a glass metabolism chamber, the formula for unfiltered radiation does not apply accurately but this method was followed for two reasons. It was found better to avoid handling the bird as much as possible and the ventilation in the metabolism chamber prevented the accumulation of ozone which might, in itself, have affected the metabolism.

#### Results.

Very little is known about the resting metabolism of birds. Only two papers were found on the subject, one by Regnault<sup>6</sup> in 1849, and another by Groebbel's<sup>7</sup> in 1920. Regnault gives the following results: Green finch, 13.00 grams, parrot 10.97 grams, sparrow 9.595 grams of oxygen per kilogram of bird per hour. For canary birds Groebbel's found 114.6 milligrams of oxygen, per bird, per 30 minutes.

Before exposure to X-rays, a series of measurements were carried out in order to establish the normal resting metabolism of canaries. The results are given in Table 1.

Using the formulae:

$$\sigma = \text{Standard deviation} = \sqrt{\frac{\sum d^2}{N}}$$

$$\text{Probable error of Mean} = .67449 \frac{\sigma}{\sqrt{N}}$$

I found for my results—

$$\sigma = \sqrt{\frac{77.8651}{45}} = \sqrt{1.73034} = 1.3154$$

$$\text{P. E. Mean} = .67449 \frac{1.3154}{\sqrt{45}} = .1323$$

$$\text{Mean} = 8.45 \pm .1323 \text{ grams per kilogram of bird per hour} \\ \text{(the weight of the bird including feathers).}$$

TABLE I.

Bird	Oxygen Grams per Kilogram of Bird per hour N. T. P.	d Deviation from the Mean	d <sup>2</sup>
I	8.68	.23	.0529
	9.71	1.26	1.5876
	8.68	.23	.0529
	7.89	.56	.3136
	7.26	1.19	1.4161
	7.34	1.11	1.2321
	7.57	.88	.7744
	7.89	.56	.3136
	8.59	.14	.0196
	9.68	1.23	1.5129
	9.37	.92	.8464
	8.73	.28	.0784
	7.34	1.11	1.2321
II	7.18	1.27	1.6129
	6.95	1.50	2.2500
	6.33	2.12	4.4944
	7.10	1.35	1.8225
III	8.98	.53	.0529
	8.08	.37	.1369
	7.63	.82	.6724
	9.06	.61	.3721
IV	8.51	.06	.0036
	7.96	.49	.2401
	7.11	1.34	1.7956
	5.86	2.59	6.7081
	5.86	2.59	6.7081
V	6.39	2.06	4.2436
	6.00	2.45	6.0026
	9.25	.80	.6400
	9.31	.86	.7396
	7.98	.47	.2209
	8.29	.16	.0256
	9.63	1.18	1.3924
VI	9.24	.79	.6241
	9.48	1.03	1.0609
	10.27	1.82	3.3124
	10.81	2.36	5.5696
	10.96	2.51	6.3001
	10.04	1.59	2.5281
	10.50	2.05	4.2025
	10.04	1.59	2.5281
	9.11	.66	.4356
	9.73	1.28	1.6384
	9.34	.89	.7921
	8.73	.28	.0784
Total	380.44	-----	77.8651

Mean = 8.45

After establishing the resting metabolism of the normal birds, they were then exposed to the X-rays and given the following doses:

Bird I received 2 skin doses April 6th, 2 skin doses April 20th, and 4 skin doses April 27th (see Fig. II).

Bird II received 2 skin doses on April 8th, and died on April 10th (see Fig. III). During the exposure the bird turned in the metabolism chamber so that its legs were exposed and it showed lesions on the legs at the time of death.

Bird III died before exposure to X-rays.

Bird IV received 1 skin dose on April 25th and died on the 28th with no apparent lesions (see Fig. III).

Bird V had  $\frac{1}{2}$  skin dose on May 3rd and was observed until May 14th (see Fig. IV).

Bird VI had 4 skin doses on May 6th and was observed until May 14th (see Fig. IV).

The results of the X-ray exposures are shown graphically in Figures II, III and IV, and are given numerically in Table II. The last column in Table II shows the maximum percentage of variation from the normal mean for each bird for a short period of a few hours immediately following the exposure, and the average percentage of variation over a longer period of several days or a week following exposure. This column shows at once that, although the immediate effect was a sharp increase in resting metabolism, this increase was followed by a depression which was very marked for heavy doses, and which lasted a week or more. Only with a dose as small as  $\frac{1}{2}$  skin dose was the average metabolism above normal during the week after radiation.

### CONCLUSIONS.

The experiments show that after exposure to moderate doses of X-rays there is a marked, though short-lasting, increase in the rate of oxygen consumption. For a dose of  $\frac{1}{2}$  skin unit this increase amounted to 24.36 per cent. over the normal, for 1 skin dose there was an increase of 34.2 per cent., and for 2 skin doses there was an increase of 50.3 per cent. (average for Bird I, Dose I and Bird II, Dose I).

A second radiation of 2 skin doses, on the same bird, resulted in a small increase of 17.8 per cent. (Bird I, second dose).

The increase always came on immediately after exposure, the maximum coming from 20-25 minutes after exposure, and the



metabolism returned to normal in about an hour. After this short period of stimulation the mean resting metabolism was lower than it had been before radiation except in the case of  $\frac{1}{2}$  skin dose. The bird that had one skin dose showed an average depression of 12.26 per cent. for three days after the initial stimulation had passed off. Bird I, after its first radiation of 2 skin doses, showed a depression averaging 7.5 per cent. over a period of two weeks. After its second dose it showed a depression of 10.8 per cent. for a week and after a third dose, which amounted to four skin doses, there was no initial stimulation at all. The immediate effect was a depression of 24.4 per cent. The next day the metabolism was nearly normal but showed an average depression of 9.9 per cent. for several days. This bird was not observed any further so that it is not known how long it was before the metabolism came back to its original value. A new bird, given four skin doses, showed a small rise of 9.3 per cent. followed by a gradual and steady fall, which gave an average decrease of 19.7 per cent. for a week.

Further work on other animals with X-rays of different penetrations should be made but the results with canaries after exposure to soft X-rays lead one to the following conclusions: A dose of  $\frac{1}{2}$  skin unit gives a small stimulation to the resting metabolism with no subsequent depression. Doses of 1 to 2 skin units give a marked temporary stimulation followed by a slight depression which lasts for a week or more. A dose of four skin units may give a small initial rise in metabolism but the subsequent depression is very marked. These results fall in line with the well-known fact that small doses of X-rays are in general stimulating, whereas large doses are injurious.

In conclusion, I wish to thank Dr. J. H. Clark and Dr. A. L. Meyer for the interest they have taken in my work and for their kind assistance in accomplishing it.



GRAMS OXYGEN PER KILOGRAM BIRD PER HOUR (N.T.P.)

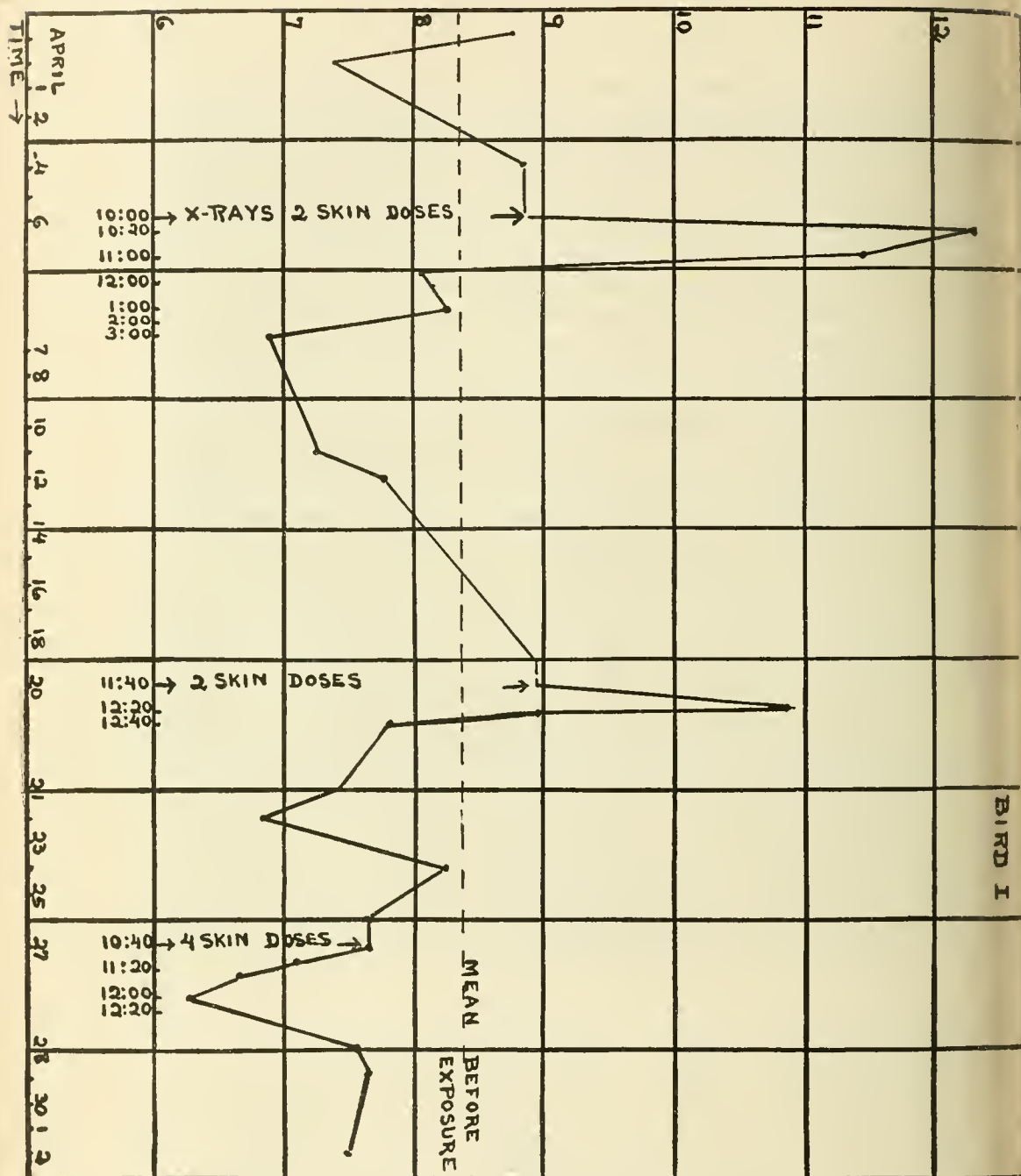


Fig. II—Effect of three X-ray exposures on the resting metabolism of bird I.

Exposure I and II=2 skin doses.

Exposure III=4 skin doses.

GRAMS OXYGEN PER KILOGRAM BIRD PER HOUR (N.T.R)

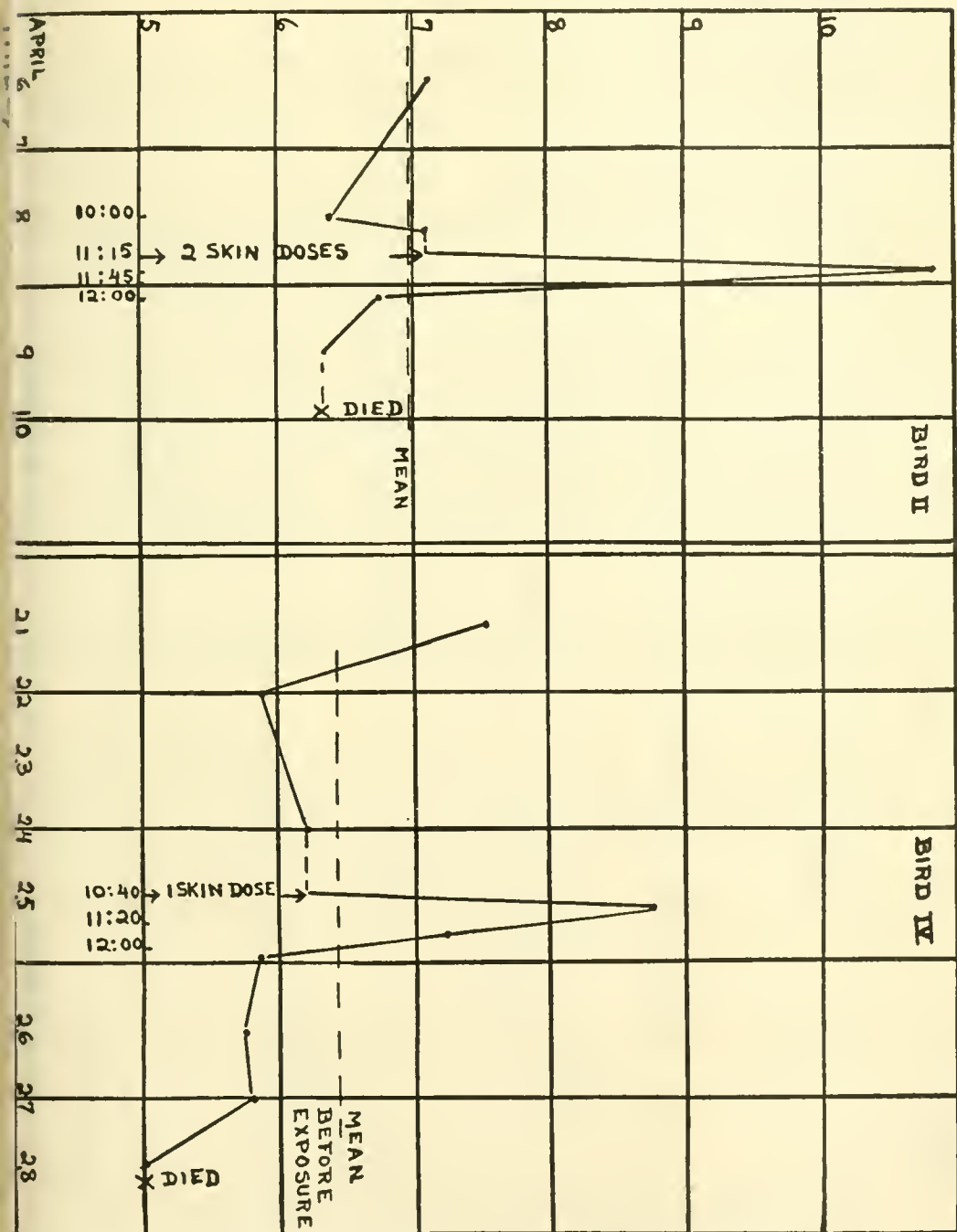


Fig. III—Effect of 2 skin doses on the resting metabolism of bird II and 1 skin dose on the resting metabolism of bird IV.

## GRAMS OXYGEN PER KILOGRAM BIRD PER HOUR (N.T.P.)

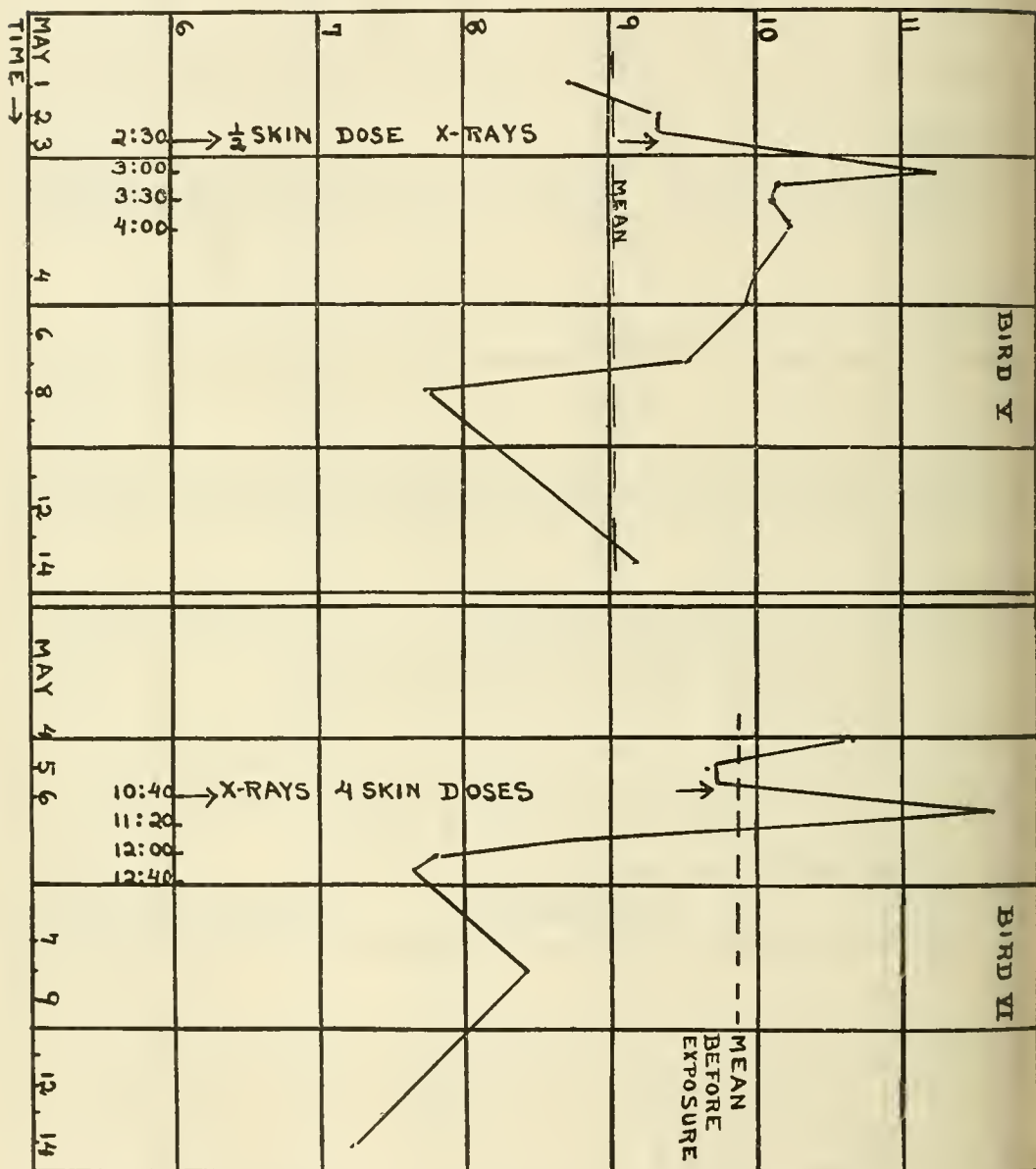


Fig. IV.—Effect of ½ skin dose on the resting metabolism of bird V and 4 skin doses on the resting metabolism of bird VI.

## REFERENCES.

1. Moleschott, J. *Wien. Med. Woch.* 5, 1855, 681.  
Martin, H. N., and Friedenwald, J. *Studies from Biol. Lab. J. H. U.*, 1887, p. 221.
2. Huldshinsky, K. *Ztschr. f. Kinderheilk.*, 26, 1920, 207.  
Hess, A. F., and Unger, L. J. *J. Amer. Med. Assn.*, 77, 1921, 39.  
Hess, A. F., and Gutman, P. *Proc. Soc. Exper. Biol. and Med.*, 19, 1921, 31.  
Powers, G. F., Park, E. A., Shipley, P. G., McCollum, E. V., and Simmonds, N. *J. Amer. Med. Assn.*, 78, 1922, 159.  
Park, E. A. *Physiol. Rev.*, 3, 1923, 106.
3. Williams, O. T. *Biochem. Journ.*, 1906, vol. I, p. 249.
4. Kikkoji. *Radium in Biol. u. Heilkunde.*, 1, 1911, 46.  
Thies, R. C., and Bagg, H. J. *J. Biol. Chem.*, 41, 1920, 525.  
Redfield, A. C., and Bright, E. M. *J. Gen. Physiol.*, 4, 1922, 297.
5. Meyer, A. L. *Amer. J. Physiol.*, 65, 1923.
6. Regnault, and Reiset, J. *Annales de Chem. et de Physique*, ser. 3, T. 26, 1849.
7. Groebbels, F. *Ztschr. f. Biol.*, 70, B 1920, 477.





## FURTHER STUDIES ON METABOLISM AFTER EXPOSURE TO X-RAYS.

J. H. CLARK, P. S. EVANS, JR., AND  
A. PENA CHAVARRIA.

*Johns Hopkins School of Hygiene and Public Health.*

The results of Barreto's work on the resting metabolism of canary birds after X-ray exposure (1) were of a somewhat preliminary nature and it was impossible for him to continue the work. These results have since been confirmed by the authors of this paper, and certain points brought out in these subsequent experiments are of sufficient interest to warrant their publication as a supplement to Barreto's paper.

### *Method.*

Meyer's method for determining the basal metabolism of small animals was used (2), and Barreto's procedure followed, except that the birds were exposed in an open box covered only with mosquito netting, instead of in the metabolism chamber. The two female canaries used had been worked with at intervals throughout the winter. Their normal variations in metabolism were known and they were accustomed to the experiment. A new bird often shows too high a value for the resting metabolism until it becomes used to being handled. After determining the normal resting metabolism of these birds for a period of two weeks, they were exposed to X-rays and the metabolism determined a number of times during the next hour in order to find the exact time at which the subsequent rise occurred.

The X-ray dosage was calculated from Remer and Witherbee's formula for unfiltered rays.

$$\frac{\text{Spark gap} \times \text{milliamperes} \times \text{time}}{(\text{distance})^2} = \frac{1}{r^2} = 1 \text{ skin dose.}$$

In the first exposure of Bird I the secondary voltage was 70,000 volts, which is equivalent to a five inch spark gap. In all subsequent exposures 55,000 volts or a three and a half inch spark gap was used.

### *Results.*

The results are given in Table I and in Fig. 1.

TABLE I.

Time	Grams of oxygen per kilogram bird per hour N. T. P.	Respirations per minute	Weight (grams)	Remarks
Bird I.				
Mar. 15	10.22	-----	16.58	-----
" 22	10.36	-----	15.92	-----
" 27 (2:15)	10.02	-----	15.95	Before X-ray
2:35 (X-rays)	-----	-----	-----	2 skin doses at 2:35
2:40	9.34	-----	-----	5 min. after Radiation
3:00	11.49	-----	-----	25 " " "
3:45	9.24	-----	-----	1 hr. 10 min. after Radiation.
Mar. 29	9.06	-----	-----	-----
April 10 (2:15)	8.24	126	15.28	Before X-ray
2:25 (X-rays)	-----	-----	-----	2 skin doses at 2:25
2:40	9.50	120	-----	15 min. after X-ray
2:50	10.964	112	-----	25 " " "
3:10	10.09	114	-----	45 " " "
3:45	8.95	114	-----	1 hr. 20 min. after X-ray
April 12	9.6	114	14.3	-----
April 17 (2:20)	8.19	120	15.28	Before X-ray
2:35 (X-rays)	-----	-----	-----	4 skin doses at 2:30-2:35
2:40	7.73	114	-----	5 min. after X-ray
3:00	10.22	120	-----	25 " " "
3:40	9.9	116	-----	1 hr. 5 min. after X-ray
4:15	8.99	118	-----	1 " 40 " " "
April 19	7.56	94	14.36	-----
Bird II.				
April 5	7.62	-----	18.944	-----
" 12 (2:15)	7.596	-----	18.48	Before X-ray
2:40 (X-rays)	-----	-----	-----	4 skin doses at 2:30-2:40
2:45	7.697	-----	-----	5 min. after X-ray
3:00	7.3	-----	-----	20 " " "
3:50	6.7	-----	-----	1 hr. 10 min. after X-ray
April 17	7.02	178	17.88	-----
April 24 (3:15)	7.44	184	17.28	Before X-ray
3:30 (X-rays)	-----	-----	-----	4 skin doses at 3:25-3:30
3:35	7.09	176	-----	5 min. after X-ray
3:55	7.70	174	-----	25 " " "
4:15	7.67	180	-----	45 " " "

The experiments on Bird 1 show clearly that two skin doses of unfiltered X-rays (spark gap 3.5-5 inches) results in a small drop in the resting metabolism five minutes after exposure. This small drop is followed by a sharp rise which reaches a maximum twenty-five minutes after the exposure. After this sharp rise there is a return to normal in about an hour, or an hour and a half, and then a steady fall over a period of one to two weeks accompanied by a fall in weight. The normal resting metabolism of Bird 1 had been in the neighborhood of 10 grams of oxygen per kilogram of bird per hour throughout the winter, and its weight had varied between 16 and 17 grams during that time, so that the drop in

weight and metabolism following X-ray exposure is more significant than might be supposed at first glance. No explanation can be offered for the sharp rise in metabolism after exposure, but it is certainly not associated with any rise in the rate of respiration which stays practically constant. (See Table 1.)

The results for four skin doses are less satisfactory. Barreto found in one bird a slight rise followed by a more marked depression than usual and in another bird no rise, only the subsequent depression. Our results on Bird II, exposure I, agree with his in showing only a depression after radiation but Bird I responded to four skin doses with a rise which is practically the same as that following two skin doses except that the maximum seems to come later, about thirty to thirty-five minutes after exposure judging from the shape of the curve.

The return to normal is much slower, for the metabolism is well above its pre-exposure value an hour and three quarters after radiation. This bird unfortunately was not studied more than two days after this last exposure and so it is not known whether a long period of depression followed this rise or not. The results for four skin doses are obviously unsatisfactory but those for two skin doses are very consistent and not only confirm Barreto's result but also strengthen his evidence for a period of depression following the initial stimulation. The steadiness of the rate of respiration during these changes in metabolism, and the fall in weight accompanying the fall in metabolism are new points not brought out in Barreto's paper.

#### REFERENCES.

1. Barreto, A. L. B. *This journal*, preceding paper.
2. Meyer, A. J. *Amer. Journ. Physiol.*, 65, 1923.

# GRAMS OXYGEN PER KILOGRAM BIRD PER HOUR (N.T.R.)

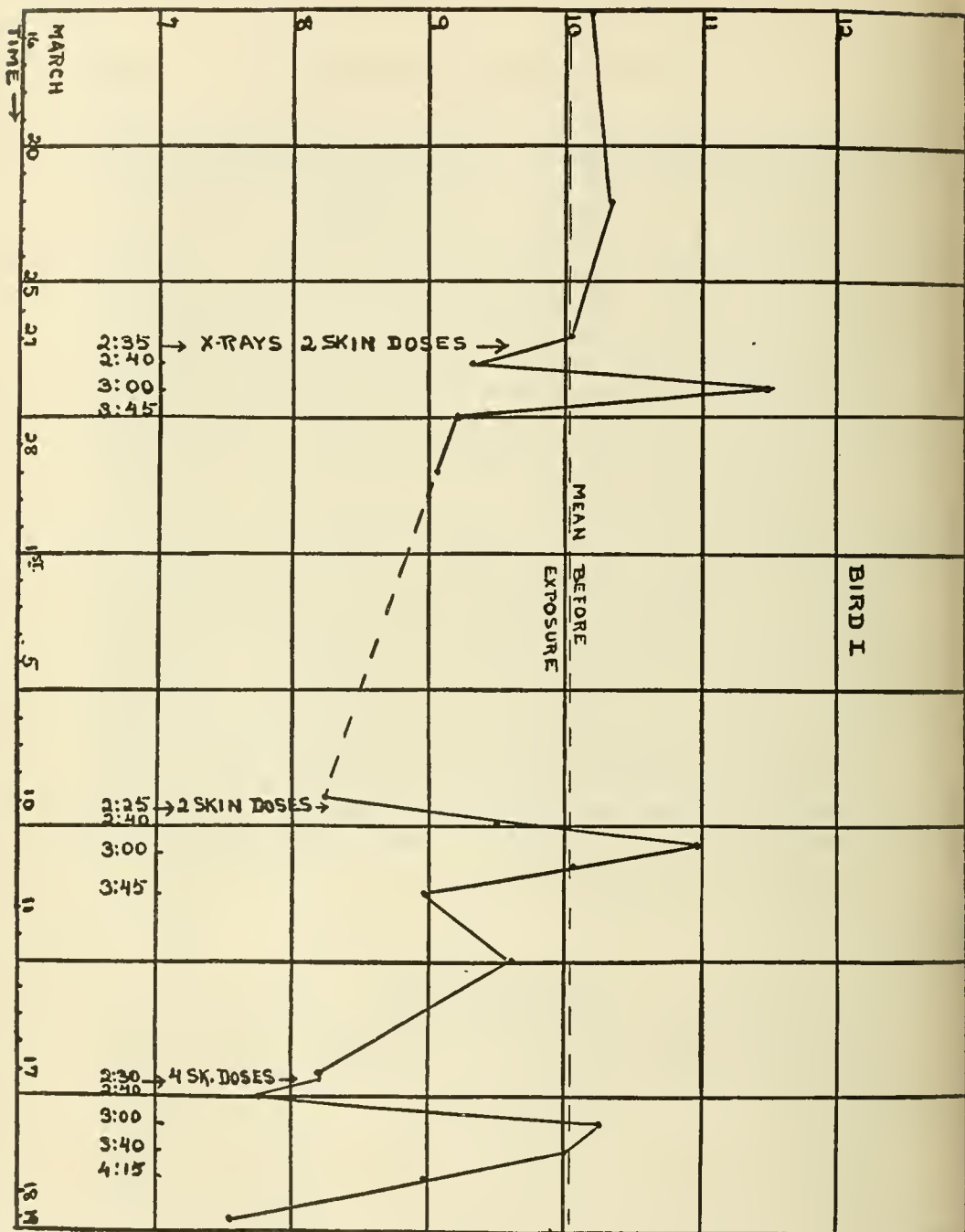


Fig. 1. Effect of three X-ray exposures on the resting metabolism of Bird I.

Exposure I=2 skin doses (70,000 volts).

Exposure II=2 skin doses (55,000 volts).

Exposure III=4 skin doses (55,000 volts).

## A COMPARISON OF DODDS' AND SLADDEN'S METHODS FOR ESTIMATING URINARY DIASTASE.

BY GORDON CAMERON, M.B., B.S. (Melbourne)

*Stewart Lecturer in Pathology, Melbourne University, Victoria, Australia.*

Perhaps one of the most certain signs of the increasing importance of urinary diastatic estimations is the description of modifications in technique of the original Wohlgemuth method within the last year or so. During 1922, two such modifications were described, one by Dodds,<sup>1</sup> the other by Sladden.<sup>2</sup> Both set out with the same idea, viz. to standardize urines to a constant H ion concentration and then to perform the estimation as in the manner of Wohlgemuth,<sup>3</sup> but each attacked the problem from a slightly different standpoint. It occurred to the author that a series of comparisons of the three methods, Wohlgemuth's, Dodds' and Sladden's, might be of interest and of practical value in deciding which method should be adopted.

### *Method.*

The Wohlgemuth technique is known to all, being described in most text books of clinical medicine.

Dodds standardizes urines to the optimum reaction,  $\text{pH}=6.1$ , for urinary diastase in the presence of phosphates, by diluting the urines to be tested with a phosphate buffer solution, obtained by mixing 15 cc. of Sørensen's solution (A), with 85 cc. of solution (B). Solution A is made by dissolving 11.876 gm. of  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  in one litre of boiled distilled water, the solution being kept in a paraffin coated bottle. Solution B is made by dissolving 9.078 gm. of  $\text{KH}_2\text{PO}_4$  in one litre of boiled distilled water and stored in a paraffin coated bottle. The resulting solution, obtained as above, should have a  $\text{pH}$  of 6.1. The diastatic value is then obtained in much the same way as in the Wohlgemuth method, the calculation being the same. Sladden standardizes urines to the optimum reaction  $\text{pH}=6.7$ , for diastase in the presence of chloride by treating with deci-normal  $\text{NaOH}$ , using phenol red as indicator, the first appearance of a faint pink tinge being taken as the end point corresponding to a  $\text{pH}=6.7$ . With alkaline urines titration back with deci-normal  $\text{HCl}$  may be necessary. Sladden omits the addition of a phosphate buffer mixture on the ground that it tends to obscure the reading of final results.

In this investigation 65 urines have been examined, in each case the diastatic value by the three methods being determined.



*Results.*1. *Diseases of the Kidney.*

Diagnosis	Values of Diastase (Units)		
	Wohlgemuth's Method	Sladden's Method	Dodds' Method
Pyonephrosis .....	2.8	2.8	2.8
Pyonephrosis .....	4	6.7	6.7
Pyelitis .....	3.3	5	5
Chronic Nephritis .....	2.5	2.8	4
Chronic Nephritis .....	20	20	20
Chronic Nephritis .....	5	5	6.7

2. *Circulatory Diseases.*

Cardiac Failure .....	10	10	10
Cardiac Failure .....	2.5	2.8	3.3
Cardiac Failure .....	2.5	2.8	3.3
Cardiac Failure .....	2.5	3.3	5
Cardiac Failure .....	2.2	2.2	2.5
Cardiac Failure .....	10	10	10
Cardiac Failure .....	6.7	10	10
Cardiac Failure .....	22.2	22.2	22.2
Cardiac Failure .....	10	20	20
Cardiac Failure .....	10	10	10
Cardiac Failure .....	10	20	22.2

3. *Diseases of the Lungs.*

Pulmonary Tuberculosis .....	3.2	3.2	4
Pneumonia .....	4	6.7	6.7
Pneumonia .....	4	10	10
Pneumonia .....	20	25	25
Bronchopneumonia .....	22.2	22.2	22.2
Pneumonia .....	2	2.2	3.3
Pneumonia .....	5	5	5
Bronchopneumonia .....	4	5	6.7

4. *Diseases of the Liver and Gall Bladder.*

Cholelithiasis .....	5	6.4	6.4
Cholelithiasis .....	10	33.3	20
Cholelithiasis .....	10	10	10
Cholelithiasis .....	40	50	50
Cholecystitis .....	6.7	20	22.2
Cirrhosis of Liver .....	10	10	10
Cirrhosis of Liver .....	25	28.5	28.5
Cirrhosis of Liver .....	25	33.3	33.3

5. *Diseases of the Pancreas.*

Chronic Pancreatitis .....	66.7	100	100
Carcinoma of Head of Pancreas	28.5	33.3	33.3
Carcinoma of Head of Pancreas	1	1	1
Chronic Pancreatitis .....	66.7	100	100
Subacute Pancreatitis .....	66.7	100	100
Diabetes Mellitus .....	6.6	10	10
Diabetes Mellitus .....	4	4	4

6. *Diseases of Stomach and Intestines.*

Gastric Ulcer .....	3.2	4	4
Intestinal Obstruction .....	2.5	2.8	2.8
Dysentery .....	10	10	20
Typhoid Fever .....	10	20	22.2
Carcinoma of Stomach .....	10	20	20
Gastric Ulcer .....	20	20	20

7. *Diseases of Thyroid Gland.*

Exophthalmic Goitre .....	10	20	20
Exophthalmic Goitre .....	2.8	10	10
Exophthalmic Goitre .....	10	10	10
Exophthalmic Goitre .....	10	10	10
Exophthalmic Goitre .....	20	20	20
Parenchymatous Goitre .....	10	25	22.2

8. *Diseases of Joints.**Diagnosis.*

Rheumatic Fever .....	4	4	6.7
Rheumatoid Arthritis .....	20	20	20
Rheumatoid Arthritis .....	10	10	10
Rheumatoid Arthritis .....	5	20	20
Rheumatic Fever .....	5	6.7	5
Rheumatoid Arthritis .....	20	20	25
Rheumatoid Arthritis .....	5	10	10

9. *General.*

Tonsilitis .....	5	10	6.7
Tonsilitis .....	10	20	20
Cerebro-spinal syphilis .....	10	10	10
Chyluria .....	30	40	33.3
Cerebral tumor .....	10	10	10

*Discussion.*

The value of the methods may be seen by considering the ratios

$$\frac{\text{Values obtained by Sladden's Method}}{\text{Value obtained by Wohlgemuth's Method}} = \frac{S}{W},$$

$$\frac{\text{Value obtained by Sladden's Method}}{\text{Value obtained by Dodds' Method}} = \frac{D}{S}.$$

$$\text{The value of the first expression ie. } \frac{S}{W} = 1.398,$$

$$\text{the value of the second expression ie. } \frac{D}{S} = 1.005.$$

Hence we see that on an average the value obtained by Dodds' Method differs from that obtained by Sladden's Method by 5 parts in 1000 ie. .5%, whereas the value obtained by Sladden's Method differs from that obtained by Wohlgemuth's technique by nearly 400 parts in 1000 ie. 40%

For practical purposes we may conclude that there is no appreciable difference in the values obtained by Sladden's and Dodds' Methods. As Dodds' Method is slightly easier to carry out and has less chance of any personal error, its adoption should be urged.

Incidentally, this investigation demonstrates certain principles:

1. The diastatic value in kidney diseases is generally subnormal.
2. In inflammatory conditions of the pancreas, the diastatic value is greatly increased above normal.
3. In malignant disease of the pancreas, the value is within normal limits, occasionally being subnormal.
4. In diabetes mellitus, the value is either normal or subnormal.

All of this work was carried out at the Walter and Eliza Hall Institute of Research, through the kindness of Dr. S. W. Patterson.

## REFERENCES

1. Dodds, E. C. *Brit. J. Exper. Path.*, June, 1922.
2. Sladden, A. F. *Lancet*, July 8, 1922.
3. Wohlgemuth, J. *Berl. klin. Woch.*, 39, 1910, 302, 324.





# NON-PROTEIN NITROGEN AND BLOOD PRESSURE IN RELATION TO KIDNEY AND HEART LESIONS.

BY ROLFE FLOYD, M. D.  
New York City.

The purpose of this paper is to show certain relationships between non-protein nitrogen and blood pressure on the one hand, and structural changes in the kidney and heart on the other. It is based on fifty cases in which the non-protein nitrogen was determined during life and on which I performed autopsies and examined the organs microscopically. In only two-fifths of these cases was nephritis the chief, or an important contributory cause of death.

As so often happens in writing up routine hospital cases, the observations are neither so well controlled nor as complete as desirable. Thus the blood pressure and the heart and kidney weights were not always recorded; the blood pressure and non-protein nitrogen observations were taken at varying and sometimes considerable intervals before death; and so on. In spite of these imperfections, the work is presented because more observations on the relation of clinical findings to lesions are essential to the solution of the present kidney problems.

In the kidney microscopical changes are far more significant than gross ones, so in this study only the following structural alterations are considered:

1. Changes in the combined weight of the two kidneys. The normal figure has been considered to be from 270 to 300 gm. for the adult.
2. The occurrence of an inflammatory exudate in the form of pus cells, red blood cells, coagulated matter and casts as they occur in the lumina of the tubules and of the glomeruli or, less commonly, in the interstices of the stroma. The exudate has been described as (a) absent, (b) slight, (c) marked, and (d) very great. It is obvious that such a classification is both ill-defined and too dependent on the personal equation, although the latter error is somewhat controlled by a rather wide experience in kidney sections. The same division into four degrees of change is followed in dealing with other kidney and heart lesions.

3. Degeneration of the epithelium of the convoluted tubes consisting of swelling, distortion, granulation, vacuolization and desquamation. (Necrosis did not appear in this series.) These changes are difficult to judge of because postmortem changes occur early, probably within six hours, and closely simulate those which occur before death. In most kidneys brought to microscopical study, therefore, postmortem effects are present, and often antemortem phenomena in addition. I have recorded the changes as seen but, except in early autopsies or marked lesions, such observations must be interpreted with reserve.
4. Changes in glomeruli which include an increase in the tuft cells obscuring the capillary loops, matting of the loops, waxy degeneration of the loops, increase of the capsule cells to form crescents, thickening of Bowman's capsule, shrinkage and atrophy of the entire structure with obliteration of the tuft.
5. Increase of connective tissue stroma, in subcapsular wedges, in irregular patches or diffusely, varying in relative amounts of cells and of basement substance.
6. Thickening of the arteries, presenting the ordinary or obliterating type of endarteritis.

Structural changes in the heart are considered under four heads:

1. Changes in heart weight. The organ was freed of its contents before weighing.
2. Changes in the heart valves: thickening, distortion, vegetation and destruction.
3. Changes in the heart muscle consisting of areas of degeneration and necrosis, areas of fibrosis, areas of infiltration with pus or round cells.
4. Thickening of the coronary arteries, similar to the endarteritis seen in the kidney and elsewhere.

#### *The Relation of Non-Protein Nitrogen to Kidney Changes.*

The non-protein nitrogen was found to vary more directly with the amount of stroma in the kidney cortex than with any other renal lesion.

There was almost no relation between non-protein nitrogen figures and changes in the renal epithelium. There was, however, a definite tendency for the non-protein nitrogen and the amount of the exudate to vary together and the same proved true in regard to glomerular and arterial changes.

In other words an increase of non-protein nitrogen goes with an increase of the inflammatory exudate in the kidney, an increase of glomerular lesions and an increase of arterial thickening, but its increase corresponds most closely with an increase in the connective tissue while there seems to be very little relation between it and the amount of epithelial degeneration.

The significant relation of kidney weight to non-protein nitrogen was the increase of average non-protein nitrogen in small kidneys, a result to be anticipated since such organs are the ones that show the maximum obliteration of tubules and glomeruli. In the table normal non-protein nitrogen figures occur with high kidney weights, but this result is based on four cases, one of which had waxy kidneys weighing 660 gm. In two others, however, the kidney weights were large, 500 gm. and 380 gm. respectively, but the organs without other significant abnormality. Whether these large normal kidneys represent a hypertrophy in response to some physiological demand which has resulted in an increase of the normal functional power must be determined by further studies. Such a condition occurs at any rate in the heart when first put under continued strain. Also I believe that those who drink large amounts of beer have been observed to have large kidneys without inflammatory changes.

Some of the detailed results are here tabulated to support the above statements and to enable other observers to include them in larger series of observations. In looking at this and subsequent tables the following average figures for the entire series must be constantly kept in mind.

		No. of Observations
Average kidney weight.....	329 gm.....	43
Average heart weight.....	448 gm.....	35
Average blood pressure.....	153 mm.....	38
Average N P N.....	116 mg. in 100 cc.....	50

*Relation of Non-Protein Nitrogen to Kidney Weight.*

N P N	Average Kidney Weight	No. of Observations
Up to 30 mg.....	460 gm.....	4
31-50 mg. ....	322 gm.....	13
51-100 mg. ....	312 gm.....	9
101-200 mg. ....	321 gm.....	8
Over 200 mg.....	293 gm.....	9

*Relation of Non-Protein Nitrogen to Inflammatory Exudate in the Kidney.*

Exudate	Average N P N	No. of Observations
No exudate .....	44 mg.....	12
Slight exudate .....	98 mg.....	13
Marked exudate .....	167 mg.....	17
Very large exudate.....	145 mg.....	8

*Relation of Non-Protein Nitrogen to Degeneration of the Renal Epithelium.*

Epithelium	Average N P N	No. of Observations
Normal .....	43 mg.....	9
Slight degeneration .....	132 mg.....	18
Marked degeneration .....	133 mg.....	23

*Relation of Non-Protein Nitrogen to Glomerular Changes.*

	Average N P N	No. of Observations
Glomeruli normal .....	35 mg.....	7
Slight glomerular lesions.....	92 mg.....	14
Marked glomerular lesions.....	142 mg.....	19
Extreme glomerular lesions.....	158 mg.....	10

*Relation of Non-Protein Nitrogen to Increase of Connective Tissue in the Kidney.*

Stroma	Average N P N	No. of Observations
Normal .....	56 mg.....	19
Slight increase .....	61 mg.....	11
Marked increase .....	191 mg.....	9
Extreme increase .....	215 mg.....	11

*Relation of Non-Protein Nitrogen to Thickening of Renal Arteries.*

	Average N P N	No. of Observations
Normal arteries .....	80 mg.....	22
Slight thickening of arteries.....	97 mg.....	11
Marked thickening of arteries....	163 mg.....	14
Extreme thickening of arteries..	239 mg.....	3

At the first glance thickening of the renal arteries seems to correspond to a higher non-protein nitrogen than does stroma increase, but the higher figure, 239 mg., occurring with extreme arterial change is based on only three cases. If the cases showing little and no change are compared with those showing marked and extreme change in each series the greater correspondence of non-protein nitrogen with variation in the stroma becomes at once apparent—thus:

	Average N P N	No. of Observations
Little or no stroma change.....	58 mg.....	30
Marked or extreme stroma change	240 mg.....	20
Little or no arterial thickening.....	85 mg.....	33
Marked or extreme art. thickening	148 mg.....	17

It is interesting in this connection to recall that Delafield, who saw a great many autopsies on cases which had been under his observation during life, defined chronic nephritis as a disease whose essential and characteristic lesion was the growth of new connective tissue in the kidneys, although his work was done without knowledge of the non-protein nitrogen.

Volhard and Fahr likewise found that "glomerulo-nephritis," in which an increase of the stroma is early and important, was associated with higher non-protein nitrogen figures than either "nephrosis" or "sclerosis" in which the increase of stroma is less pronounced and of later development.

The reason for this elevation of non-protein nitrogen figures as the stroma increases is, I believe, the corresponding absence or deterioration of tubes and glomeruli. Whether the increase of connective tissue is looked at as the primary fact in the inflammation or as a replacement fibrosis secondary to primary destruction of the secretory structures, the ultimate result is the same. A kidney section that shows a lot of connective tissue must contain comparatively fewer secretory elements. If the whole organ



were over-size due to an increase of connective tissue without any concurrent destruction of the tubes and glomeruli then the above view would not hold, but I have never seen such an organ showing no damage to the secretory elements.

*Relation of Non-Protein Nitrogen to Heart Lesions.*

The heart weight shows a definite tendency to increase with the non-protein nitrogen, probably incident to the rise of blood pressure that, as a rule, occurs with increasing nitrogen in the blood.

On the other hand no definite relationship was found between various lesions of the valves, myocardium and coronary vessels and variations in non-protein nitrogen figures. The tabulated results follow:

*Relation of Heart Weight to Non-Protein Nitrogen.*

N P N	Average Heart Weight	No. of Observations
Up to 30 mg.....	268 gm.....	3
31-50 mg. ....	427 gm.....	12
51-100 mg. ....	473 gm.....	4
101-200 mg. ....	472 gm.....	8
201 mg. and over.....	503 gm.....	8

This relation of heart weight to non-protein nitrogen is not a vital one for it fails to come out when the table is reversed so as to show the average non-protein nitrogen that occurs with progressively increasing groups of heart weights.

*Relation of Non-Protein Nitrogen to Heart Weight.*

Heart Weight	Average N P N	No. of Observations
Up to 300 gm.....	49 mg.....	7
301-400 gm. ....	126 mg.....	9
401-500 gm. ....	210 mg.....	7
Over 500 gm.....	152 mg.....	12

The failure of non-protein nitrogen to continue its progressive increase when the heart weight reaches maximum figures is an interesting observation whose significance will be discussed later in the paper in considering the relationship of non-protein nitrogen to blood pressure.

*Relation of Non-Protein Nitrogen to Heart Valves.*

Heart Valves	Average N P N	No. of Observations
Normal .....	144 mg.....	23
Slight lesions .....	108 mg.....	18
Marked lesions .....	73 mg.....	4
Extreme lesions .....	49 mg.....	4

Here the non-protein nitrogen varies inversely with the condition of the valves; that is, the more abnormal the valves the more normal the non-protein nitrogen. While this result would probably not hold for a larger series of cases, it certainly indicates that a poor circulation and chronic congestion of the kidney interfere little with the power of the kidney to excrete nitrogenous waste.

*Relation of Non-Protein Nitrogen to Myocardial Changes.*

Myocardium	Average N P N	No. of Observations
Normal .....	106 mg.....	40
Slight lesions .....	233 mg.....	3
Marked lesions .....	204 mg.....	3
Extreme lesions .....	54 mg.....	2

These results only indicate that myocarditis was an infrequent lesion in this series and that when it occurred it exerted no controlling influence on the non-protein nitrogen.

*Relation of Non-Protein Nitrogen to Coronary Thickening.*

Coronaries	Average N P N	No. of Observations
Normal .....	113 mg.....	21
Slight thickening .....	116 mg.....	15
Marked thickening .....	252 mg.....	5
Extreme thickening .....	54 mg.....	2

Here again there is no evidence of a significant relationship.

*Relation of Systolic Blood Pressure to Changes in the Kidneys.*

The blood pressure was taken in thirty-eight of the fifty cases, so the conclusions are based on twelve less observations than in the cases of the non-protein nitrogen. When several readings were taken the highest only is used in my tables.

The outstanding relationship between blood pressure and kidney changes is its correspondence with the thickening of the renal arteries, a result made doubly striking by the fact that it does not correspond at all with the thickening of the coronary arteries. This concordance of blood pressure with renal arteritis is in accord with the work of Volhard and Fahr on renal sclerosis.

The tabulated results follow:

*Relation of Blood Pressure to Kidney Weight.*

Kidney Weight	Average Blood Pressure	No. of Observations
Up to 270 gm.....	179 mm.....	12
270-300 gm. ....	142 mm.....	6
Over 300 gm.....	144 mm.....	15

The somewhat higher average with small kidneys goes with the fact that arterial changes are apt to be maximum in atrophic organs. The same fact is brought out later in the table which shows that the arteries are thicker in kidneys of light weight.

*Relation of Blood Pressure to Inflammatory Exudate in the Kidney.*

Exudate	Average Blood Pressure	No. of Observations
None .....	123 mm.....	7
Slight .....	151 mm.....	13
Marked .....	144 mm.....	11
Very large .....	197 mm.....	7

*Relation of Blood Pressure to Degeneration of the Renal Epithelium.*

Epithelium	Average Blood Pressure	No. of Observations
Normal .....	145 mm.....	7
Slight degeneration .....	153 mm.....	15
Marked degeneration .....	157 mm.....	16

*Relation of Blood Pressure to Glomerular Changes.*

Glomeruli	Average Blood Pressure	No. of Observations
Normal .....	118 mm.....	5
Slight lesions .....	132 mm.....	8
Marked lesions .....	159 mm.....	16
Extreme lesions .....	181 mm.....	9

*Relation of Blood Pressure to Increase of Connective Tissue Stroma.*

Stroma	Average Blood Pressure	No. of Observations
Normal .....	119 mm.....	16
Slight increase .....	172 mm.....	5
Marked increase .....	155 mm.....	8
Excessive increase .....	200 mm.....	9

*Relation of Blood Pressure to Thickening of Renal Arteries.*

Arteries	Average Blood Pressure	No. of Observations
Normal .....	121 mm.....	14
Slight thickening .....	155 mm.....	8
Marked thickening .....	180 mm.....	12
Extreme thickening .....	206 mm.....	3

This relative correspondence of various kidney lesions with blood pressure is more sharply brought out in a table showing the average blood pressure in relation to normal structure and slight lesions grouped together and compared with the average blood pressure in marked and extreme lesions similarly grouped.

	Epithelium	Exudate	Glomeruli	Stroma	Arteries
Normal structure and slight lesions	150 mm.	143 mm.	126 mm.	132 mm.	133 mm.
Marked and extreme lesions	157 mm.	165 mm.	167 mm.	179 mm.	185 mm.

These figures show an interesting progression of correspondence between increasing kidney lesions on the one hand, and increasing blood pressure figures on the other. Epithelial changes stand at the bottom of this scale and arterial changes at the top. While it bears out Volhard and Fahr's conclusions about sclerosis of the renal arteries, it does not support their view that the glomerulus is to be regarded simply as a part of the vascular mechanism of the kidney.

*Relation of Blood Pressure to Changes in the Heart.*

The correspondence between increase in blood pressure and increase in heart weight is most evident. Hardly any other result could be credited because blood pressure is perhaps the most potent factor in cardiac enlargement in adult life. On the other hand variations in blood pressure cannot be correlated with changes in the valves and myocardium.

It is surprising, however, that thickening of the coronary arteries does not go with high blood pressure in this series, the more surprising since thickening of the renal vessels and increasing blood pressure closely correspond.

*Relation of Average Blood Pressure to Heart Weight.*

Heart Weight	Average Blood Pressure	No. of Observations
Up to 300 gm. ....	112 mm.....	5
301-400 gm. ....	157 mm.....	4
401-500 gm. ....	135 mm.....	5
Over 500 gm. ....	189 mm.....	12

*Relation of Average Heart Weight to Blood Pressure.*

Blood Pressure	Average Heart Weight	No. of Observations
Up to 120 mm. ....	350 gm.....	8
121-150 mm. ....	416 gm.....	5
151-200 mm. ....	571 gm.....	7
Over 200 mm. ....	590 gm.....	6

These two tables show that the effect of blood pressure on heart weight is more definite than that of heart weight on blood pressure, and this result is to be expected, because high blood pressure almost always makes the heart big, but big hearts are caused by other things than high blood pressure.

*Relation of Average Blood Pressure to Lesions of the Heart Valves.*

Valves	Average Blood Pressure	No. of Observations
Normal .....	162 mm.....	21
Slight lesions .....	144 mm.....	11
Marked lesions .....	120 mm.....	1
Extreme lesions .....	146 mm.....	4

The instances of severe lesions are few, but it is obvious that valve lesions do not necessarily increase blood pressure.

*Relation of Average Blood Pressure to Myocardial Lesions.*

Myocardium	Average Blood Pressure	No. of Observations
Normal .....	154 mm.....	29
Slight myocarditis .....	155 mm.....	3
Marked myocarditis .....	148 mm.....	3
Extreme myocarditis .....	157 mm.....	2



This table gives no evidence of any influence of myocarditis on blood pressure, and, were it not for the small number of cases showing myocardial lesions, would indicate that none exists.

*Relation of Blood Pressure to the Thickening of the Coronary Arteries.*

Coronaries	Average Blood Pressure	No. of Observations
Normal .....	143 mm.....	16
Slightly thickened .....	172 mm.....	10
Moderately thickened .....	151 mm.....	5
Extremely thickened .....	157 mm.....	2

This lesion does not go with an elevation of blood pressure in this series.

By leaving out of account the number of observations, the different effect of renal and coronary sclerosis on blood pressure can be compared as follows:

*Comparison of the Relation of Coronary Sclerosis and of Renal Sclerosis to Blood Pressure.*

Lesions	Average Blood Pressure with Coronary Artery Lesions	Average Blood Pressure with Renal Artery Lesions
Normal vessels .....	143 mm.....	121 mm.
Slight sclerosis .....	172 mm.....	155 mm.
Marked sclerosis .....	151 mm.....	180 mm.
Extreme sclerosis .....	157 mm.....	206 mm.

This result is very convincing and should induce further studies on the relation between local scleroses and blood pressure. There is a general idea that arteritis and blood pressure should go together, but there are many exceptions to this rule. It has not been my impression that thickened cerebral arteries have a very definite relation to blood pressure, and the whole subject needs more understanding.

*Other Relations of Symptoms and Lesions.*

*Eye ground changes.* The eye grounds are apt to be looked at only when a change is expected and so in this series their condition is recorded in only nineteen cases, including thirteen cases of nephritis and two of brain tumor.

Five of the nephritics showed definite neuroretinitis, while five other nephritics had entirely normal eye grounds. In comparing these two groups, both presented about the same degree of change

in the kidney stroma and in the renal arteries. The cases with normal grounds showed rather more albumin in the urine and a higher average non-protein nitrogen figure, 247 mg. as compared with 197 mg. The only striking difference between the two groups was in the blood pressure, an average of 245 mm. in the nephritic cases with neuroretinitis contrasted with an average of 161 mm. in those without. The series is entirely too small to form any judgment. The result is only given so that it can be included in larger series of other workers.

*Relation of Albumin in the Urine to the Exudate in the Kidney.*

This is obviously a comparison that rests on peculiarly uncertain data, for on the one hand it is the junior or substitute interne who examines the urine, while on the other hand to judge how much exudate exists throughout both kidneys by how much is seen in one section cannot give reliable results. In this series there is shown, however, a rough correspondence between the exudate in the urine during life and the kidney after death. No case with no albumin in the urine showed an excessive exudate in the tubes, and every case with a urine that boiled solid showed some exudate in the kidney. In general the cases with smaller amounts of albumin in the urine showed less exudate in the kidney than those with larger amounts of albumin. More than this cannot be said.

There is, however, in addition, an apparent connection between the amount of albumin in the urine and the average kidney weight; thus, in nineteen cases, with no albumin or a trace only, the average weight of the kidney was 313 gm., while in fifteen cases showing a precipitate, or boiling solid the average weight was 371 gm. It is not improper to infer that an exudate within the kidney in part explains this difference, especially as there is no significant relation of albumin in the urine to other kidney changes, i.e., to epithelial, glomerular, stromal or arterial lesions. It is worth noting that Volhard and Fahr's contention, that epithelial degeneration is the basic cause of albumin in the urine, is not borne out, for in this series of cases there was a complete absence of relationship between these two phenomena.

*Dropsy* in this series shows little tendency to vary in degree with the amount of kidney lesion or with the changes in any one element of the kidney more than another, except that epithelial changes seem to have less relation to it than other kidney lesions.

*Dyspnea*, on the other hand, tends more definitely to increase with increasing kidney lesions, least with epithelial and most with arterial changes.

The relation of symptoms to each other does not require autopsies for its study and a small series such as the present is fruitless for general purposes. It may be stated, however, that the non-protein nitrogen and blood pressure show some, but not a striking, tendency to vary together. The smaller increases of non-protein nitrogen over normal in this series go with a progressive increase of blood pressure, but as the non-protein nitrogen rises to higher levels the blood pressure fails to maintain a corresponding increment. Thus:

*Relation of Average Blood Pressure to Non-protein Nitrogen.*

N. P. N.	Average Blood Pressure	No. of Observations
Up to 30 mg. ....	127 mm.....	4
31-50 mg. ....	136 mm.....	14
51-100 mg. ....	173 mm.....	6
101-200 mg. ....	165 mm.....	6
Over 200 mg. ....	171 mm.....	8

There seems to be an explanation of this failure of blood pressure to follow the higher non-protein nitrogen figures in the two kinds of nitrogen retention. The first kind of uremia is due to a gradual failure of the power of the kidney to get rid of the nitrogen which enter the blood in twenty-four hours. This demands a stronger physiological stimulus in order to maintain a nitrogen balance and this stimulus is supplied by a greater concentration of non-protein nitrogen in the blood. This accounts for the elevated non-protein nitrogen figures which often remain comparatively constant for weeks or months. This process seems to go hand in hand with the loss of glomeruli and tubules, and the corresponding increase of stroma in the kidney. This lesion is regularly accompanied by a thickening of the renal arteries with which an increase of the blood pressure goes along as has been indicated above. So it happens that up to a certain point we should expect this corresponding increase of non-protein nitrogen and blood pressure which has been noted by every observer of kidney disease.

There is, however, a second type of uremia and one which usually becomes controlling in the very high non-protein nitrogen cases. This second type depends on the inability of the kidney

to concentrate the solution of urea and other nitrogenous waste products in the urine. This power weakens rather rapidly under strain, so that ordinary volumes of urine are insufficient to carry off the twenty-four hour waste, and the residue is retained in the body and, in part, piles up in the blood. This process advances rapidly and yields high non-protein nitrogen figures within a week or ten days and causes a uremic death from true retention. It occurs in damaged kidneys after sudden strains such as surgical operations and the like, and is often a final and fatal complication in a chronic nephritis. It causes the high and fatal non-protein nitrogen figures but not, so far as I know, an increased blood pressure. Thus it happens that moderate and persistent elevations of non-protein nitrogen are apt to be associated with progressive increases of blood pressure, while rapid, very high and terminal elevations of non-protein nitrogen are not associated with corresponding elevations of blood pressure. This is so in this series and I believe it will prove substantially true for larger series also.

The relation of lesions to each other also needs only a large series of autopsies independent of clinical data for its study. Certain observations may be worth recording, however.

The kidney weight varied inversely with the amount of new connective tissue and with the thickening of the renal vessels, in other words, as the stroma increases and the vessels thicken there is probably a loss of the secretory elements of the kidney which overbalances the gain in stroma and which is not compensated for by an increase in exudate. This presumably goes along with a loss in volume although this was not studied in this series.

*Relation of Average Kidney Weight to Increase of Stroma.*

Stroma	Average Kidney Weight	No. of Observations
Normal .....	327 gm.....	14
Slight increase .....	307 gm.....	9
Marked increase .....	343 gm.....	8
Great increase .....	285 gm.....	12

*Relation of Average Kidney Weight to Thickening of Renal Arteries.*

Renal Arteries	Average Kidney Weight	No. of Observations
Normal .....	377 gm.....	17
Slight thickening .....	354 gm.....	10
Marked thickening .....	256 gm.....	12
Extreme thickening .....	240 gm.....	3

Epithelial changes, on the other hand, which almost always result in a swelling of the cells, do not in this series induce a definite and progressive gain in the kidney weight.

*Relation of Average Kidney Weight to Degeneration of the Epithelium.*

Epithelium	Average Kidney Weight	No. of Observations
Normal .....	348 gm.....	6
Slight changes .....	280 gm.....	17
Marked changes .....	364 gm.....	20

Increasing amounts of exudate, as estimated from the study of the sections, while showing some correspondence with increasing kidney weights do not yield a convincing relationship.

*Relation of Average Kidney Weight to Exudate in the Kidney.*

Exudate	Average Kidney Weight	No. of Observations
None .....	304 gm.....	9
Slight .....	312 gm.....	11
Marked .....	362 gm.....	15
Extreme .....	318 gm.....	8

The heart weight, which reveals chiefly the mass of cardiac muscle, did not vary as much with the disease of the valves or with coronary thickening as with changes in the kidney, especially with thickening of the renal vessels. This last relation was to be anticipated because of the above discussed relations of blood pressure to renal vessels and to heart weight respectively.

*Relation of Average Heart Weight to Valve Changes.*

Heart Valves	Average Heart Weight	No. of Observations
Normal .....	477 gm.....	18
Slight valvulitis .....	402 gm.....	10
Marked valvulitis .....	341 gm.....	4
Extreme valvulitis .....	573 gm.....	3

*Relation of Average Heart Weight to Coronary Thickening.*

Coronary Arteries	Average Heart Weight	No. of Observations
Normal .....	379 gm.....	17
Slight thickening .....	509 gm.....	10
Marked thickening .....	528 gm.....	4
Extreme thickening .....	390 gm.....	1



*Relation of Average Heart Weight to Thickening of Renal Arteries.*

Renal Vessels	Average Heart Weight	No. of Observations
Normal .....	355 gm.....	15
Slight thickening .....	434 gm.....	6
Marked thickening .....	539 gm.....	12
Extreme thickening .....	645 gm.....	2

The relation of heart weight to kidney weight shows only a moderate tendency for the heart to increase in size as the kidney weight departs from normal either up or down.

*Relation of Average Heart Weight to Kidney Weight.*

Kidney Weight	Average Heart Weight	No. of Observations
Normal: 270-300 gm.....	409 gm.....	7
Less than normal .....	457 gm.....	13
Greater than normal .....	437 gm.....	14

## SUMMARY

The data in this paper indicate (1) that an increase in non-protein nitrogen is more closely associated with an increase in the kidney stroma than with any other of the renal lesions here studied, and (2) that an increase of blood pressure and of heart mass are more closely associated with thickening of the renal arteries than with any other lesion here studied, whether in the heart or kidney.

The results are founded on data too few in number and too poorly controlled to justify anything approaching a final judgment. The work is put forward as a contribution to present kidney problems.

## EXPERIMENTAL STUDIES IN DIABETES.

### SERIES V. ACIDOSIS.

#### 1. The Production of Diabetic Acidosis and Coma in Dogs.

FREDERICK M. ALLEN, M. D.

*From the Hospital of the Rockefeller Institute for Medical Research,  
New York.*

One of the chief arguments against the identity of clinical and experimental diabetes in the past has been the difference in respect to acidosis, viz., that the patient with severe diabetes typically dies in coma while the depancreatized dog dies of wasting and weakness. Some of the literature on this topic was previously discussed.<sup>1</sup> The greater susceptibility of man to acidosis as compared with the dog and most lower species must be recognized. Nevertheless the identity of the two forms of diabetes is best substantiated by proof that coma can result from the same conditions in both. The reports of rare ketosis or doubtful coma in totally depancreatized dogs never sufficed to settle the doubts. The pancreatic atrophy in the chronic form of diabetes studied by Sandmeyer<sup>2</sup> and by Langfeld<sup>3</sup> likewise hinders fat digestion, so that such dogs never die in coma and their slight ketonuria has but slight experimental value. The most favorable opportunity is afforded by the type of partially depancreatized dogs which retain satisfactory power of fat digestion. The production of coma in diabetic dogs thus becomes a regular routine matter, provided that certain conditions of diet are fulfilled which are ordinarily present in human cases. Single examples will serve as well as many. Therefore types of results by the two successful methods will first be given, followed by the negative observations in totally depancreatized dogs and remarks upon various causes of failure.

It is well established that high fat diets in the presence of active diabetic symptoms constitute the surest means of inducing coma in man, and the same fact holds for dogs. Fasting, though it causes a slight ketonuria in normal persons, generally tends to reduce severe acidosis and ward off coma. Nevertheless some

TABLE 1  
Dog B2-52

Date	Body Weight kg.	URINE					BLOOD PLASMA				
		Vol. cc.	Sugar %	Sugar gm.	Total N gm.	Nitro- prusside	Ferric Chloride	Sugar mg. per 100 cc.	Nitro- prusside	CO <sub>2</sub> Cap. Vol. %	
1914											
Dec. 21	14.3	256	3.0	7.68	.....	+	0	362	0	49.8	
" 22	.....	125	2.1	2.62	.....	+	0	.....	.....	.....	
" 23	.....	130	2.4	3.12	.....	+	0	.....	.....	.....	
" 24	.....	89	2.8	2.49	.....	+	0	.....	.....	.....	
" 25	.....	165	5.2	8.58	.....	+	0	414	+	43.6	
" 26	12.9	106	2.2	2.33	.....	+	0	.....	.....	.....	
" 27	.....	372	2.7	10.04	.....	+	0	.....	.....	.....	
" 28	.....	102	2.6	2.65	.....	+	0	.....	.....	.....	
" 29	.....	110	1.9	2.09	.....	++	0	.....	.....	.....	
" 30	12.25	230	1.3	2.99	4.7	++	0	381	+	39.2	
" 31	.....	174	1.8	3.13	3.6	++	+	.....	.....	.....	
1915											
Jan. 1	.....	219	1.6	3.50	3.9	++	+	.....	.....	.....	
" 2	.....	230	1.4	3.12	4.4	++	+	.....	.....	.....	
" 3	.....	*950	1.2	11.40	4.8	+	0	.....	.....	.....	
" 4	.....	*1347	1.0	13.47	5.2	++	0	393	++	29.4	
" 5	10.3	40	2.6	1.04	0.6	++	+	372	++	12.7	

\*Mixed with vomited water.

patients, particularly those with some obesity and susceptibility to acidosis, are actually thrown into coma by fasting, and this rule also applies to dogs when the conditions are imitated. This less frequent condition will be described first.

### 1. *Fasting Acidosis.*

The early record of dog B2-52 was given previously.<sup>4</sup> This female bull terrier, weighing 12.8 kg. in a state of very good nutrition, was partially depancreatized on April 24, 1914. The tissue removed weighed 20.4 gm., and the remnant about the main duct was estimated at 3.1 gm. (about  $\frac{1}{8}$ ). Heavy feeding with bread and glucose gave only transitory glycosuria. The attempt to produce diabetes in this way failed when the dog lost appetite for the mixture and fell in weight to 9.3 kg. In this condition on May 18, approximately half of the pancreas remnant, weighing 1.4 gm., was removed. This tissue was microscopically normal, except for doubtful thinning of cytoplasm in some island cells.

Glycosuria followed this operation, but was checked by three days of fasting. A diet of 100 to 300 gm. of beef lung was then given until June 5, by which time the weight was reduced to 8.6 kg. The tolerance had thus been increased so that only traces of glycosuria resulted from 1,200 gm. of beef lung, and on diets between 1,000 and 1,200 gm. the body weight rose slowly to 9.1 kg. on June 14; 9.8 kg. on July 1; 10.4 kg. on July 17, and 11.4 kg. on July 30. Glycosuria appeared to the extent of 0.53 per cent. sugar in 507 cc. urine on July 30, and 1.6 per cent. in 836 cc. urine on July 31. Fasting was begun August 1. The glycosuria fell immediately to traces but did not cease until August 10, indicating a very stubborn hyperglycemia. August 12, with sugar-free urine and body weight of 9.25 kg., a diet of 100 gm. lung was begun. This was gradually increased so that by August 22 the dog was taking 500 gm. lung without glycosuria, at a weight of 9.0 kg. September 5, the weight was 8.9 kg. and the diet was increased to 600 gm. lung, which was also tolerated without glycosuria. September 11, when the weight was 8.8 kg., 50 gm. lard was added to the diet. The weight gradually rose, without glycosuria or ketonuria, to 10.5 kg. on October 5, when the diet was increased by 50 gm. lard, making 600 gm. lung and 100 gm. lard. The fat, with the usual small quantities of bone meal and talcum powder, was always well eaten, but the lung ration was kept mostly at 600 gm., because larger quantities were eaten for only a few days. November 25 to 28, at a weight of 12.5 kg., glycosuria of 0.4 to 2.0 per cent. was present, but ceased with a single fast day on November 29.

December 7, 0.36 per cent. sugar appeared in 645 cc. urine. The nitroprusside reaction was also positive and remained so. The dog at this time weighed 14.3 kg., and appeared in splendid strength and spirits. Though the animal might have passed anywhere as a thriving normal dog, the glycosuria quickly rose to 3 and then to 5 per cent.

December 21, fasting was begun in the attempt to stop the glycosuria, and not with any intent of producing acidosis. The chemical observations

are shown in Table 1. The glycosuria diminished somewhat but showed no signs of clearing up. Ketonuria was persistent but evidently not heavy; the total acetone excretion must have been trivial. The dog was strong, lively and hungry at first, but toward the close became quiet and slightly dejected, and showed decreasing interest in food. By January 1 and 2 all food offered was refused. On January 3 and 4 the dog was nauseated, occasionally vomited clear mucus, and was very desirous of drinking large quantities of water, which were very soon vomited. Weakness progressed rapidly at this stage, and death occurred in profound prostration on January 5, consciousness being apparently clear to almost the last moment, though the dog was insensitive to pain as usual in this condition.

The usual picture of fasting acidosis in man was thus duplicated in all details. The clinical features are nausea, profound weakness and retention of consciousness. The ketonuria was evidently much lower than in man. The acetone retention indicated by nitroprusside tests of the plasma became heavy only at the end. The plasma bicarbonate was at first nearly normal for a dog, but fell rapidly at the close and before death reached a minimum of 12.7 volumes per cent.

Dog B2-80 has also been mentioned in former publications.<sup>5,6</sup> This was a black and white female mongrel, weighing 17 kg. in good nutritive condition. On November 17, 1914, 25.7 gm. of pancreatic tissue was removed, leaving a remnant about the ducts estimated at 7.1 gm. ( $\frac{1}{4}$ - $\frac{1}{5}$ ). There was a tendency to glycosuria with this large pancreas remnant, and by the feeding of bread with as much as 500 gm. of glucose daily the diabetes was finally made permanent. This diabetes was kept under control in such manner that in May the tolerance was approximately 1 kg. of lung, at a body weight generally between 14 and 15 kg. With rise of weight to 15.5 kg., glycosuria developed on this diet at the end of May. By fasting and reduction of weight to 13.2 kg. early in June, the diet of 1 kg. of lung was temporarily tolerated, until on June 9, 0.17 per cent. sugar appeared in 681 cc. urine, followed by heavier glycosuria the next day. After a fast day, a diet of 500 gm. lung and 300 gm. suet was tolerated until June 21. The cause of the decline of tolerance was a rise of weight to 17 kg.

After another fast day, the diet was changed to 250 gm. lung and 300 gm. suet. The weight thus rose to 18 kg., and the dog acted and appeared as a splendidly healthy animal, as shown by the previously published photograph,<sup>7</sup> which was taken under these conditions on July 2.

On July 10, the weight reached the maximum point of 18.75 kg., and 1.4 per cent. sugar appeared in 605 cc. urine. The glycosuria ceased with two fast days. Experiments with exercise were then begun, as previously described.<sup>6</sup> Some reductions of plasma sugar were thus obtained, but not enough to prevent the return of glycosuria. Slight glycosuria and also ketonuria continued through most of this period, and were not perceptibly altered by the exercise, though the animal was strong and willing and ran to exhaustion in the treadmill.



TABLE 2  
Dog B2-80

Date 1915	Weight kg.	URINE						BLOOD					Diet
		Total N gm.	NH <sub>4</sub> N gm.	Sugar gm.	D/N Ratio	Nitro- prusside	Ferric Chloride	Plasma Sugar mg. per 100 cc.	Hgb. %	Nitro- prusside	CO <sub>2</sub> Cap. Vol. %	Lipemia Qual.	
July 28	17.5	.....	.....	Faint	.....	+	0	.....	.....	.....	.....	.....	500 gm. lung.
" 29	17.5	.....	.....	8.79	.....	+	0	.....	.....	.....	.....	.....	Not fed.
" 30	17.3	.....	.....	Neg.	.....	+	0	.....	.....	.....	.....	.....	200 gm. suet.
" 31	17.4	.....	.....	"	.....	+	0	.....	.....	.....	.....	.....	250 gm. lung.
Aug. 1	.....	.....	.....	Faint	.....	+	0	.....	.....	.....	.....	.....	250 "
" 2	.....	.....	.....	6.95	.....	+	0	.....	.....	.....	.....	.....	250 "
" 3	.....	.....	.....	7.11	.....	+	0	.....	.....	.....	.....	.....	Not fed.
" 4	.....	.....	.....	2.84	.....	+	0	.....	.....	.....	.....	.....	"
" 5	.....	.....	.....	1.01	.....	+	0	.....	.....	.....	.....	.....	"
" 6	.....	.....	.....	5.74	.....	+	0	.....	.....	.....	.....	.....	"
" 7	.....	.....	.....	5.79	.....	+	0	270	91	+	.....	0	"
" 8	.....	.....	.....	4.48	.....	+	0	294	102	+	.....	0	"
" 9	15.7	.....	.....	5.23	.....	+	0	263	98	+	.....	0	"
" 10	.....	.....	.....	5.40	.....	+	+	286	112	+	.....	0	"
" 11	.....	.....	.....	13.75	.....	+	+	222	97	+	.....	0	"
" 12	.....	3.07	0.93	6.35	2.07	+	+	345	70	+	21.8	+	"
" 13	14.5	15.21	3.66	37.92	2.50	+	+	327	95	+	24.2	+	"
" 14	.....	7.19	0.50	7.66	1.06	+	+	435	70	+	29.0	+	"

The later record is shown in Table 2. The fast beginning August 3 was imposed not with a view to producing coma, but in the attempt to clear up glycosuria by the combination of fasting and exercise. The diabetes had formerly been controlled by brief fasts, but owing to the prolonged overnutrition, it had finally reached a hopeless stage. The sugar excretion fell to approximately 1 gm. on August 5, but then increased, indicating the hopeless condition. The dog remained apparently well clinically, and was able to run hard on the last exercise day, August 8, though the tendency to dyspnea was increased. There was no benefit and likewise no apparent harm from this exercise; the signs of acidosis seemed not to be increased chemically or clinically. Except for the continuous glycosuria and moderate ketonuria, conditions continued seemingly normal until August 11, when nausea and occasional vomiting of fluid began. On August 12, this symptom was worse and weakness and dyspnea were evident. The asthenia was manifest by the fact that the dog remained lying down while drinking water. Water was quickly vomited, and all food was refused. Slight exertion brought on prostration and dyspnea. A dose of 5 gm. of sodium bicarbonate was given by stomach tube, but it is doubtful if any was retained. The rectal temperature was 103° F. As a precaution against dryness, 1,200 cc. of 0.85 per cent. sodium chloride solution was injected subcutaneously.

August 13, weakness and dyspnea were still more prominent, and increasing stupor appeared during the day. Doses of sodium bicarbonate given by stomach or through a long tube by rectum were not retained. Therefore 5 gm. of bicarbonate in 200 cc. of water was injected subcutaneously, and sufficed to prevent further fall of the plasma bicarbonate. The clinical condition was not improved. The rectal temperature was 102.5° F.

In the early morning of August 14, the dog became comatose so that attempts at arousing elicited only feeble cries followed by a relapse into sleep. The conjunctival reflex remained present. Painful intestinal cramps seemed to occur at intervals, as indicated by cries and contraction of abdominal muscles. The breathing was deep and stertorous, like true coma. Only a few cc. of urine were obtainable by catheter, and in the last few hours of life there was complete anuria. A dose of 5 gm. of sodium bicarbonate by stomach tube was partly retained, because stupor lessened the tendency to vomit. An intravenous injection of 400 cc. of 0.85 per cent. sodium chloride solution had no clinical effect. The eyeballs were very soft, as in human coma. During the last hour or two of life unconsciousness was complete; the animal could not be roused to any response, except that the conjunctival reflex was retained to the end. Death occurred at 4 P. M., and autopsy was performed immediately.

The gross findings were entirely negative except for a very large fatty liver. Abundance of body fat was still present. The pancreas remnant, entirely normal in appearance and consistency, weighed 13.5 gm. The microscopic study has been previously reported;<sup>8</sup> the outstanding feature was the complete absence of islands, except for probable small groups of surviving alpha cells.

A post-mortem blood sample contained 23 mg. urea and 559 mg. NaCl per 100 cc. of plasma. Chemical tests for glycogen in liver and muscles were negative. Samples of fresh tissues were thoroughly minced in cold water and repeatedly extracted. The combined filtrates gave the following results in comparison with the blood:

	Blood	Liver	Muscle
Acetone + acetoacetic, mg. per 100 cc.....	1.4	1.0	1.35
Beta-oxybutyric (as acetone) mg. per 100 cc.....	32.3	8.65	54.0

## 2. Acidosis with Feeding.

Dog C3-27, a female yellow mongrel, weighed 16.25 kg. in a state of good nutrition.<sup>9</sup> July 8, 1915, pancreatic tissue weighing 34 gm. was removed, leaving a remnant about the main duct estimated at 4.4 gm. (1-9). Mild diabetes resulted at first, so that glycosuria readily occurred on bread diet. On a protein-fat diet the weight rose as high as 21 kg. by the close of February, 1916, but notwithstanding this obesity there was no return of glycosuria on the feeding of bread and as much as 400 gm. glucose daily. This was one of the examples of cure of diabetes by hypertrophy of the pancreas remnant.

March 9, additional pancreatic tissue weighing 0.9 gm. was removed. This tissue was entirely normal in acini and islands. The feeding of bread and glucose then caused glycosuria, and the tolerance was broken down so that the sugar excretion remained heavy, first on bread and then on mixed diet. Beginning March 27, the diet was 200 gm. beef lung, 100 gm. bread and 100 gm. suet. The weight was 18.75 kg. On April 1, the bread was reduced to 50 gm., and on April 8 the suet was increased to 200 gm. Increasing acetonuria developed without clinical symptoms, Lipemia was also intense, so that the plasma at all times in the 24 hours resembled thick cream. It was determined that the fat obtainable from the plasma by simple ether extraction amounted to 10 per cent. or more, but no more accurate analyses were performed. The previously published photograph<sup>10</sup> was taken under these conditions on April 15, when the weight was 19 kg. The chemical observations during the terminal period have also been published elsewhere,<sup>11</sup> and the table is reproduced here.

Appetite and digestion had been maintained exceptionally well, but after April 15 the dog began to refuse suet, which had to be forcibly fed. The remainder of the diet was eaten readily after the suet was thus given. With the aid of bone meal and talcum powder, the feces continued solid and apparently well digested up to April 20, when moderate diarrhea began. Most of the diet on this day had to be fed forcibly and vomiting forcibly restrained. The respiration was perceptibly increased in both rate and depth. Beginning depression was evident, although the animal was still fairly strong and cheerful.

April 21, a considerable part of the diet was lost by vomiting, notwithstanding precautions. The respirations at times reached a maximum of 70 per minute, with increased depth, though the dog was quiet and the weather not hot. The breath had a strong acetone odor. Consciousness

TABLE 3  
Dog C3-27

Date 1916	Wgt. † kg.	URINE					BLOOD					Diet	Remarks
		Vol. cc.	Sugar gm.	Total N gm.	Total Acetone* mg.	Ammonia Nitrogen gm.	N:NH <sub>4</sub> -N Ratio	Plasma Sugar mg. per 100 cc.	CO <sub>2</sub> Cap. Vol. %	Hgb. %	Acetone qual.	Lipemia qual.	
April 15-16	.....	922	57.33	9.082	91.3	1.259	7.22	.....	.....	.....	.....	.....	Vomiting and diarrhea fre- quent during this period.
16-17	.....	406	15.63	3.059	144.8	0.875	3.50	370	22.1	106	+	++++	Rapidly in- creasing aci- dosis symp- toms. (Coma
17-18	19.0	530	30.81	6.201	129.9	0.737	8.40	400	29.0	108	++	++++	
18-19	18.4	1186	66.22	10.081	134.0	1.257	8.00	.....	.....	.....	.....	.....	
19-20	18.2	1350	56.70	11.475	489.2	1.633	7.05	400	18.5	.....	+++	++++	
20-21	.....	1480	51.80	10.952	389.2	1.717	6.36	314	23.3	.....	.....	++++	Coma
21-22	16.0	811	17.84	4.542	210.7	.....	.....	500	21.4	.....	+++	++++	

\*Total acetone bodies as Acetone.

†Note precipitous fall.

was clear but weakness very pronounced. The dog leaned against the side of the cage in standing, staggered when compelled to walk, and lay down immediately upon being left alone.

April 22, all symptoms were increased. The dog staggered drunkenly in attempting to walk, and ended by falling flat on her side and lying there panting. Slight diarrhea continued, with an admixture of dark blood, which is regularly found in dogs with diabetic coma. Vomiting was uncontrollable, so that no fluids could be retained. By 11 A. M., the dog was practically unconscious, and by noon even the eye reflex was completely lost. Injections of 5 per cent. sodium bicarbonate solution were then given into the jugular vein. The first injection of 50 cc. seemed to have a perceptible strengthening effect, probably by reason of the increase of blood volume. The effect of further injections was progressively less. A total of 200 cc. was thus given. The last dose of 50 cc. showed no influence whatever, and death occurred at 12:40 P. M.

The gross autopsy was negative except for lipemia and fatty liver. The lower portion of the large intestine showed congestion, becoming intense as the rectum was approached. No ulceration was present anywhere, but exudation of blood through the mucous membrane seemed to be responsible for the bloody admixture of the feces. The pancreas remnant, normal in appearance and consistency, weighed 7.8 gm., and had thus approximately doubled in size since the first operation. The fatty liver weighed 790 gm., the small spleen 43 gm., the combined kidneys 130 gm. Glycogen tests of liver and muscle were negative. Microscopically, the liver was infiltrated with fat and practically glycogen-free, as only rare cells at the periphery of lobules showed a few red granules with Best's carmine. The kidneys stained for fat showed heavy infiltration, and with Best's carmine showed heavy glycogen deposits in the Henle loops. The heart muscle showed very distinct red granulation with this stain, but the skeletal muscle contained only the slightest sprinkling. The general structure of these organs and also of the thyroid and adrenals was not essentially altered. The pancreatic acini were normal and well filled with zymogen. Islands were normal in size and number, possibly even hyperplastic, but the majority of their cells were maximally vacuolated.

It may be mentioned in passing that animals with heavy lipemia may offer some opportunity for a study of renal function. Here the glomeruli and convoluted tubules were nearly free from visible fat; a few very fine globules were discoverable in the lumen of these tubules and even in the capsular space. The number and size of the globules within the tubules increased as they were followed downward. The globules were largest and most numerous in the lumen of the Henle loop, where the cells also were crammed with both fat and glycogen. The globules then diminished, so that they were nearly absent from both the lumen and cells of the larger collecting tubules. The interpretation is not conclusive but appears to favor the resorption theory, as though fat reaching the lumen of the tubules were taken up by the cells especially in Henle's loops, so that very little finally passed out with the urine.



Dog C3-83, a Dachshund mongrel, aged 2 years, weighing 9.25 kg. in a state of medium nutrition, was partially depancreatized April 20, 1916. The tissue removed weighed 21.1 gm. The remnant about the main duct was estimated at 4.3 gm. The heaviest bread and glucose feeding failed to maintain glycosuria.

May 19, the weight had fallen to 8.3 gm. The pancreas remnant was traumatized by crushing between the fingers, and fragments weighing 0.3 gm. were broken off incidentally, and proved to be normal in acini and islands.

Heavy glycosuria then resulted on plain bread and soup diet, but ceased on meat diet. A border line condition was then maintained with a diet of beef lung, sometimes with additions of suet, and with occasional changes to bread diet to produce glycosuria to lower the tolerance. The weight rose as high as 9 kg., then fell on account of reduced diet, not on account of indigestion. August 22, the weight was 8 kg., and glycosuria was present in consequence of a protein diet in excess of the tolerance. On this day, the diet was changed to 250 gm. beef lung and 150 gm. suet, and slight glycosuria persisted. Ketonuria also appeared and increased gradually, while the weight rose. The record from September 7 onward is given in Table 4.

It will be noticed that during the period up to September 22, the ketonuria, though slight, was stubborn, and persisted even when glycosuria was absent. After this date, with continuous glycosuria, the ketonuria increased. Continuance of glycosuria during the fasting period, September 26 to October 2, showed that the diabetes had reached a stage of hopeless severity. The total acetone excretion rose to a maximum of 62 mg. and then fell, while clinical symptoms of acidosis remained absent. The ensuing diet of 200 gm. lung and 200 gm. suet increased both glycosuria and ketonuria. The addition of 150 gm. bread for two days (October 6 and 7) seemed to increase the acidosis, as judged especially by the heavy nitroprusside reaction of the plasma. The bread made an excessive diet and was therefore omitted. The tendency to vomiting was slight throughout, but weight was steadily lost through progressive impairment of digestion. It became a question whether the animal would die of coma or cachexia. The dark bloody admixture in the feces was present slightly on October 11 and increased on October 12. The ketonuria on these two days, instead of being measured in milligrams, rose to 2.11 gm. and 1.57 gm. respectively. The plasma bicarbonate fell, though not as low as in other dogs. Correspondingly, dyspnea was only moderate. Extreme asthenia was present instead of unconsciousness, so that the general picture resembled the fasting form of acidosis. Treatment with levulose and salt solution injections on October 12 was unavailing. No alkali was given. The dog lost consciousness about 6 A. M. on October 13, and died an hour later.

The pancreas remnant, normal in appearance and consistency, weighed 4.6 gm. Microscopically, the islands were reduced in size and number by extensive hydropic degeneration. The acinar tissue was strictly normal

TABLE 4  
Dog C3-83

Date 1916	Wght. kg.	URINE					BLOOD PLASMA				Diet	
		Vol. cc.	Sugar gm.	Total N gm.	Acetone and Diabetic Acid mg.	B-oxy- asAcce- tone mg.	Total Acetone mg.	Plasma Sugar mg. per 100 cc.	CO <sup>2</sup> Cap. Vol. %	Acetone and Diabetic Acid mg. per 100 cc.		B-oxy- asAcce- tone mg. per 100 cc.
Sept. 7	8.85	200	6.00	3.13	39.0	46.0	85.0	233	54.1	Faint		250 gm. lung and 150 gm. suet.
8	8.61	102	Faint	3.50	2.9	8.4	11.3					250 " " 150 " "
9	8.85	110	Neg.					145	58.0			250 " " 150 " "
10		141	"		Faint							250 " " 150 " "
11	8.25	52		3.00	0.9	4.1	5.0					Not fed.
12	8.15	65	Faint	2.98	4.6	7.5	12.1					100 gm. lung.
13	8.10	80	Neg.	1.06	26.8	11.2	38.0	98	54.1	15.7	16.7	200 gm. lung.
14	8.20	445	"	4.09	33.4	24.5	57.9					250 gm. lung and 150 gm. suet.
15	8.60	46	"		Heavy							250 " " 150 " "
16	8.35	202	"	2.62	14.1	26.3	40.4	122				250 " " 150 " "
17	7.95	308	"	3.51	26.2	2.4	125					250 gm. lung and 150 gm. suet.
18	7.66	624	"	4.52	43.7	53.0	96.7	189		Neg.		250 " " 150 " "
19	7.52	658	"	3.29	52.6	39.5	92.1	161		"		250 " " 150 " "
20	7.35	150	Faint	3.01	13.5	13.5	27.0			"		250 " " 150 " "
21	7.26	130	"	1.42	10.4	11.4	21.8					250 " " 150 " "
22		118	"	1.55	Heavy			208				Not fed.
23		195	Neg.	3.41	10.1	12.5	22.6	149		8.0	16.0	200 gm. lung.
24		278	4.73	7.42	7.8	13.3	21.1			21.1	21.1	400 " " 400 " "
25		330	13.43	6.93			31.0					400 " " 400 " "
26	6.90	150	1.50	2.45				400		Faint		Not fed.
27	6.75	220	10.42	2.07			34.0	322	56.0			" " "
28	6.75	158	5.37	1.86			37.0	270	55.7	Slight		" " "
29	6.65	112	0.56	1.32			Mod.	333		10.9	9.4	" " "
30	6.40	105	0.46	1.38			62.0	312		Faint		" " "
1		162	1.39	1.46			30.0	400	58.6			" " "
2	6.40	82	2.19	1.08			31.0	400		2.9	20.0	200 gm. lung and 200 gm. suet.
3	6.70	272	6.31	4.13			54.0	352		15.0	10.0	200 " " 200 " "
4	6.75	448	10.02	3.90			46.0			31.7	17.6	200 " " 200 " "
5	6.80	520	9.78	4.06			108.0					200 " " 200 " "
6	6.75	450	9.75	4.32	Heavy			312	51.9	Slight		200 " " 200 " "
7	6.65	878	46.10	3.42	Heavy		96.6	308	55.7	Heavy		200 " " 200 " "
8		415						322	52.8	Mod.		200 " " 200 " "
9	6.55	322						286	44.3	Heavy		200 " " 200 " "
10	6.40	378	7.31	3.91				435	39.5	"		200 " " 200 " "
11	6.25	512	10.24	3.07			2.11 gm.					200 " " 200 " "
12	6.35	302	11.48	4.47			1.57					200 " " 200 " "

and showed no scarring or other evidence of the acute traumatic inflammation which initiated the diabetes.

This dog received less carbohydrate in the acidosis diet than any other of our series, because the appetite for protein and fat was unusually well retained. It was also the only animal which had not been fat at any time and which developed fatal acidosis notwithstanding cachexia. As human patients often go into coma when emaciated, this single experiment is of interest as proving that the same combination can occur in dogs.

### 3. *Totally Depancreatized Dogs.*

Although, as known from the literature, totally depancreatized dogs ordinarily excrete little acetone and die in cachexia not coma, there seemed to be reason to anticipate a more severe acidosis if the right conditions were created, particularly a greater preponderance of fat in their metabolism. The following measures were therefore tried unsuccessfully. Most of the chemical figures were unfortunately lost, like those for lipemia, but the general results were conclusive.

(a) Obesity.—As partially depancreatized diabetic dogs regularly go into coma when sufficiently fattened, the question was investigated whether the more intense diabetes following total pancreatectomy might not at least occasionally be accompanied by severe acidosis if obese animals were chosen. For this purpose, together with the lipemia work and other experiments, more than twenty dogs of unusual fatness, some of them tremendously obese, were totally depancreatized. The results as respects both lipemia and acidosis were no different than in ordinary dogs. The qualitative and quantitative acetone tests in urine and blood were never heavy, and not the slightest symptom of acidosis was ever found. Authors seem never to have taken notice of the fact that the high protein catabolism of totally depancreatized dogs may furnish some explanation of their comparative immunity to ketosis.

(b) Because of the unproved statement in German literature that carbohydrate-fed dogs are specially susceptible to acidosis when deprived of carbohydrate, several dogs known to have been on bread diet for a year or more were totally depancreatized, with no result beyond the usual trivial ketonuria. A more promising plan seemed to be to take dogs, preferably obese, and keep them on a diet of pure fat for a week, using forcible feeding if necessary in the attempt to fill their depots with fat instead of gly-

cogen. Total pancreatectomy performed after the utmost fat stuffing resulted in no more than ordinary ketosis. A still more drastic preparation was to phlorizinize fasting dogs, and then either to depancreatize while glycosuria and ketonuria were present, or to employ pure fat feeding for a few days till the glycosuria ceased and then depancreatize. The former plan might have little theoretical value even if successful, but it gave surprisingly poor results because of the rapid cachexia and death of the animals so treated. The latter plan failed because the ketonuria of phlorizinized dogs ceases with the glycosuria; it could not be maintained by fat stuffing, and the animals thus prepared showed no increased tendency to ketosis following pancreatectomy. Attempts to feed fat to dogs after total pancreatectomy proved useless because of the well-known lack of digestive power, and neither fresh minced pancreas nor commercial preparations of pancreatic enzymes helped this condition. As stated in connection with lipemia (Series IV), lecithin can be injected intravenously in larger quantities than any other lipid; and if lecithin is the "metabolic" form of fat, there seemed to be some chance of overloading the fat metabolism in this manner. The preliminary tests indicated no increase of ketosis by this means, and this attempt was therefore dropped.

(c) Exercise is recognized as a means of increasing considerably the fat combustion of a fasting animal, and was therefore tried as a means of increasing acidosis. Limitations are created in totally depancreatized animals by the asthenia, but are partially escaped by choosing the dogs which survive the operation with the best strength and without infection. Figures which have been saved from one such experiment are given in table 5.

Dog C3-76, a male mongrel in a normal nutritive state, weighing 12.75 kg., was completely depancreatized on April 13, 1916. He was exceptional in being actually playful up to April 17, but the test with exercise showed the appearance of strength to be deceptive as usual. On April 17, he exercised to the limit of his strength on the treadmill, the total running throughout the day being considerable though only a fraction of what a normal dog would do. There is no evidence that the slight increase of ketosis, as compared with April 17, was due to exercise, for the acetone excretion had also increased on the previous day at rest. In the one hour exercise test on April 18, acetone was slightly increased in the blood, evidently in connection with a smaller output in the urine as compared with the preceding and following hours at rest. The animal then weakened





rapidly without any important acidosis, and was killed that night in connection with another experiment.

A previous paper<sup>a</sup> showed that exercise augments carbohydrate utilization in dogs with the milder grades of diabetes, but lacks any demonstrable effect in the severest forms, whether following total or partial pancreatectomy. Though quantitative figures for acetone can no longer be given, it was apparent from those experiments that exercise did not cause any appreciable ketosis in normal dogs, phlorizinized dogs, or dogs with any degree of diabetes. As violent panting is supposed to reduce the blood alkali, it was the more remarkable that no serious fall in plasma bicarbonate was observed in these animals. These statements apply to totally depancreatized dogs such as C3-76, with their poor muscular strength, and also to partially depancreatized animals such as B2-80, which combined splendid running power, marked obesity, glycogen poverty due to preceding fasting with glycosuria, and diabetes of such hopeless severity that the sugar excretion was not stopped by fasting or reduced by exercise.

There is a current form of clinical advice that patients with acidosis should be kept covered warmly at rest in bed in order to reduce fat metabolism and thereby the production of acetone. This statement is generally accepted because it corresponds to the general conceptions of fat combustion and acetone formation. The application of theoretical ideas to diabetes without definite proof is risky. There has been a similar tendency to assume that increase of metabolism must impose a heavier burden upon the pancreatic island function, but Series II proved that no such general law is valid. Forssner<sup>12</sup> obtained acidosis in normal subjects by combinations of fasting, fat feeding and exercise, but he demonstrated no influence of exercise other than an indirect one through reduction of the normal glycogen supply, i. e., no increase of ketonuria due directly to the increased fat combustion of exercise. Diabetic patients, of a type who neither store nor utilize much carbohydrate, seem sometimes to be thrown into coma by an exhausting journey or other strain, but, with their weakness and low blood pressure, the impairment of circulation and elimination may afford a sufficient explanation. Warmth and bed rest are commonly useful for assisting these functions and for the patient's comfort, but there is no evidence that they have any further influence or that they are necessary therapeutic measures

when the patient's comfort does not require them. The theoretical doctrine that exercise increases ketosis in human patients may or may not be true. All that can be said is that there is no proof either way, and that the evidence in dogs is against this theory.

(d) Fever.—Infections are well known to conduce to serious acidosis in human patients. As they commonly also impair carbohydrate assimilation, this fact may perhaps explain the acidosis. The specific influence of infection or of the elevated febrile metabolism upon acidosis has never been determined. In diabetic dogs, infection or fever has little or no influence toward reducing carbohydrate utilization,<sup>13, 14</sup> and frequently causes reduction or disappearance of glycosuria due to cachexia. Correspondingly, our observations in numerous totally and partially depancreatized dogs proved that accidental infections of various kinds and degrees, ranging from subcutaneous abscesses to peritonitis, never produced or increased acidosis and seemed rather to make it diminish or disappear.

(e) Anesthesia.—Observations with ether or chloroform anesthesia, employed either incidentally or for this experimental purpose, showed no tendency toward production of any appreciable acidosis in either totally or partially depancreatized dogs.

#### *4. Failures and Precautions in Partially Depancreatized Dogs.*

As by no means all patients on improper diets go into coma, and as dogs are inherently less susceptible to ketosis than human beings, it follows that the majority of diabetic dogs fail to develop any high acidosis. A large proportion of partially depancreatized dogs show impaired nutrition from the outset, in the form of loss of appetite, indigestion, diarrhea, respiratory infections, etc., and occasionally the extreme state previously described as pancreatic cachexia.<sup>15</sup> All such animals are fit only for other experiments, not for acidosis work. Many dogs thrive and eat fat well for a time and thus develop some degree of ketosis, but digestion finally breaks down so that emaciation results instead of coma. We have many records of such failures, because they outnumber the successes. They show more or less ketosis over longer or shorter periods, followed by failure of digestion, diminution of acidosis, and death from emaciation and cachexia. Such examples of slight or moderate ketosis with severe diabetes are shown in

the previously published records of dogs 356<sup>1</sup> and D4-49.<sup>16</sup> An illustration of failure to produce severe acidosis, due to not giving enough carbohydrate in the diet, is given in the following record of dog C3-13.

Dog C3-13, a male mongrel weighing 15.2 kg. in good nutrition, was partially depancreatized on June 17, 1915. The tissue removed weighed 25.8 gm. The remnant about the main duct was estimated at 2.6 gm. (1-11). The resulting diabetes was kept under control by fasting and low diet. At a reduced weight of 11 kg., the dog was able to eat lung *ad libitum* without glycosuria, but developed glycosuria whenever 100 gm. of bread was added. Protein-fat diets then gradually increased the weight so that in February, 1916, the nutrition and strength were splendid at a weight of 16 kg., but glycosuria appeared. This was stopped by a reduction of protein and increase of fat, making the diet 300 gm. beef-lung and suet *ad libitum*. Then, from February 21 onward, heavy glycosuria and slight ketonuria were produced by the addition of 100 gm. of bread to this diet. Lard was substituted at times for the suet to suit the appetite. Diarrhea was successfully prevented by bone-meal and talcum powder.

Up to March 18, the desired result was accomplished of increasing the weight to 16.75 kg. in the presence of continuous heavy glycosuria and increasing ketonuria. The bread was then reduced to 50 gm. daily—probably a mistake, as gradual impairment of the appetite and digestion of fat ensued. By April 14, the weight had fallen to 16 kg., and the chances for coma appeared unpromising. Another mistake of judgment was then made in trying to increase the acidosis by omitting bread, changing the diet to 300 gm. lung and 400 gm. suet, feeding all the suet forcibly. The record from this point is shown in Table 6.

After only two days, the attempt with this diet was made hopeless by vomiting and diarrhea. Fasting was then tried, in the hope that coma might thus result, as the dog was still somewhat obese. The D:N ratios remained rather high (for a fasting animal), and ketonuria persisted, but by April 28 it became evident that coma would not result, as symptoms were absent and the acetone was diminishing with the decline of weight and strength.

April 28, 200 cc. of cottonseed oil was given, mixed with talcum powder. A slight increase of acetone resulted, but also diarrhea, preventing continuance of the fat feeding.

The attempt to produce coma by either feeding or fasting had thus definitely failed. The animal was therefore used for a different experiment which caused death on May 1. The pancreas remnant weighed 3.9 gm. It was grossly and microscopically normal, except for some involution of acini due to fasting, and the usual advanced hydropic destruction of islands.

The dog was well suited for the purpose and had been well prepared, and the failure to obtain coma was probably due entirely to the mistake of withdrawing bread in the attempt to produce a quick increase of acidosis. If sufficient carbohydrate and protein had been retained in the

diet and not too much fat, the appetite, digestion and weight could probably have been maintained to a point where the acidosis would have resulted in coma from either feeding or fasting.

Some negative influences may be stated briefly, without details of the lengthy experiences on which the statements are based. Acute experiments are impossible; no partially depancreatized dog ever goes into coma except after a chronic course of diabetes. The previous diet, whether carbohydrate, protein or fat for any length of time preceding the operation, is entirely immaterial. Obesity is not a guide in the choice of animals, but only the ability to become obese. This means that a pre-existing obesity is entirely negligible in producing acidosis; the theoretical expectation that obese animals subjected suddenly to severe diabetes together with fasting or fat diet should develop marked acidosis proves contrary to fact; old dogs with enormous obesity, for example, generally bear the pancreatic operation badly, eat poorly if they survive, and almost invariably turn out as failures for coma purposes. Younger dogs with voracious appetites, a fondness for fat, and a tendency to put on weight in the lazy cage life are the ones to choose. The severity of the diabetes, as indicated by D:N or other values, is not decisive; severe acidosis occurs only with severe diabetes, but the D:N ratios are not always maximal, especially with fasting, and other animals with the same or higher ratios and apparently similar nutritive state may develop little acidosis. The clearing of an existing ketosis by fasting is shown in the record of dog B2-01 previously published.<sup>17</sup> Just as in patients, so also in dogs, the reduction of acidosis by fasting is more common than its increase, therefore fasting is the most uncertain way of producing coma, and continued high fat feeding is the most reliable way. Lipemia is not a constant or essential feature. For example, among the animals above described, dog C3-27 showed unusually high lipemia from an early stage; dog C3-83 had clear plasma at all times except during digestion; dog B2-80 showed no appearances beyond ordinary digestive lipemia throughout the long period of high fat diet, but developed heavy and increasing turbidity with the onset of coma symptoms in fasting; dog B2-52 died of fasting coma without visible lipemia at any time. Glycosuria produced by epinephrin is not conducive to acidosis; on the contrary the resulting cachexia ruins the experiment. Protein and carbohy-



drate in the diet are not a hindrance to acidosis; fairly liberal quantities of them are, in fact, usually necessary for success, in order to enable the dog to take the requisite quantities of fat for the requisite time, and there is danger of failure from trying to over-balance the diet too heavily with fat. The size of the pancreas remnant is not a factor in acidosis, except that dogs with the largest remnants generally have the best digestion. Dogs with small remnants may develop satisfactory acidosis, if they happen to digest fat well, but they are no more susceptible because of the smallness of the remnant. Generally it is a mistake to leave such a small amount of pancreatic tissue, in the hope of favoring acidosis by inducing severe diabetes. As a rule, the best results are obtained by leaving as large a remnant as possible, then breaking down the tolerance with forced feeding of starch and glucose, and then keeping the animal for some time in a "border-line" state by a diet within the tolerance, but with over-feeding for a few days occasionally in order to guard against recovery from the diabetes.

The practical method of producing severe acidosis and coma in dogs is therefore as follows. The animals chosen are vigorous, voracious, and fond of fat. Diabetes is produced by an operation leaving as much pancreatic tissue as possible, with free duct communication, and the mild form is turned into the severe by over-feeding. Islands, are thus specifically destroyed by hydropic degeneration, while the acinar tissue generally hypertrophies to greater or less extent. If the appetite, digestion and general condition are satisfactorily maintained in the first few weeks following operation, it is possible to proceed to the acidosis diets without further delay. Most animals, however, are improved for this purpose by being kept in a "border-line" state for some weeks or months further, during which time they may be useful for any sort of experiments which will not impair their health. Time is thus allowed for complete convalescence, adjustment to laboratory life, and hypertrophy of the pancreas remnant. As gain of weight is conducive to acidosis, some of the best animals for this purpose have been those which have been used for demonstration of the decline of tolerance with gain of weight.<sup>4</sup> A very good preparation therefore consists in bringing the animal to a state of obesity on protein-fat diets, checking each appearance of glycosuria by reduction of protein and increase of fat,



while permitting continuous hyperglycemia. The glycosuria, which becomes inevitable under these conditions, is accompanied by mild ketonuria. About this time there is generally some flagging of appetite and digestion, which must be restored by increase of protein and introduction of some carbohydrate. Glycosuria is thus greatly increased, the tolerance breaks down still more quickly, but the nutrition is kept up by fat so as to prevent loss of weight or actually to increase the weight if possible. Typical diets for the purpose have been 200 to 400 gm. of lean meat, 100 to 200 gm. of bread, and 150 to 300 gm. of suet, depending on the size and appetite of the dog. Different kinds of fat, different methods of preparation and cooking, etc., are used as ingenuity may suggest; but with increasing ketosis the dog develops an aversion to fat, in spite of all devices. For a time the fat can be fed forcibly and the dog will then eat the rest of the diet and retain everything. As the maximum acidosis is approached, appetite is usually lost completely, digestion is more or less seriously impaired, and it may be necessary to force the entire diet and then remain with the dog for hours, distracting his attention in various ways to prevent vomiting, if possible, but constricting the esophagus forcibly for this purpose if necessary. Failure at this stage is generally due to insufficient personal attention and generally ends in emaciation and diminution of acidosis, though fasting coma may develop if the preparatory program has been carried far enough. This troublesome terminal stage of feeding, however, is brief, and if carried through successfully for a few days results in obvious symptoms of impending coma. The outcome is then certain, regardless of any diet, and with fully developed coma not even insulin can save the animal.<sup>18</sup>

The entire plan consists merely in imitating the conditions which are most conducive to coma in human patients. The first stage, namely the operation and convalescence, are designed to establish an adequate power of digesting fat; the human diabetic ordinarily has good digestion while the dog is handicapped by the loss of most of the pancreas. The remaining program consists in attempting to build up the body weight in the presence of severe diabetes, according to the old-fashioned method of treatment. The difficulties arise only from the fact that dogs are by nature less susceptible to acidosis and more subject to indigestion

than human beings. When these difficulties are overcome by suitable care, the production of typical diabetic coma in dogs becomes a routine procedure.

### Conclusion.

Partially depancreatized dogs are subject to diabetic coma similar to that of human patients under similar conditions of feeding or fasting. One of the differences between clinical diabetes and that following total pancreatectomy is thus obviated, and the accurate reproduction of all details of the clinical picture strengthens the unitarian conception of diabetes as a pure pancreatic deficiency.

### REFERENCES.

1. *Amer. J. Med. Sci.*, 153, 1917, 313-372. The role of fat in diabetes.
2. Sandmeyer, W. *Ztschr. f. Biol.*, 29, 1892, 86-114; *ibid.* 31, 1895, 12-85. Ueber die Folgen der partiellen Pancreasextirpation beim Hund.
3. Langfeld, E. *Acta med. Scand.*, 8, 1920, Fasc. I. The partial pancreatectomy.
4. *Amer. J. Med. Sci.*, 161, 1921, p. 20. Changes in assimilation by alteration of body mass.
5. *J. Exper. Med.*, 31, 1920, p. 383. Also (1), table II.
6. *Amer. J. Med. Sci.*, 161, 1921, p. 177. The effects of exercise.
7. *J. Exper. Med.*, 31, 1920, p. 608, Fig. 7.
8. *J. Metabolic Research*, 1, 1922, p. 38, Fig. 13. Hydropic degeneration of islands of Langerhans after partial pancreatectomy.
9. *J. Exper. Med.*, 31, 1920, p. 390. Effects of carbohydrate diets.
10. *J. Exper. Med.*, 31, 1920, p. 608, Fig. 8.
11. (1), table IV.
12. Forssner, G. *Skand. Arch. Physiol.*, 22, 1909, 349-392; 393-405. *Ibid.*, 23, 1910, 305-325. Ueber die Einwirkung des Nahrungsfettes auf die Acetonkörperausscheidung.
13. *Amer. J. Physiol.*, 54, 1920, 375-381. The influence of fever and intoxication.
14. Wishart, Mary B., and Pritchett, Ida W. *Amer. J. Physiol.*, 54, 1920, 382-387. Gas bacillus infections in diabetic dogs.
15. *Amer. J. Med. Sci.*, 161, 1921, 350-365. Pancreatic cachexia.
16. *J. Metabolic Research*, 3, 1923, 623-639. Diabetes and phlorizin glycosuria.
17. *J. Exper. Med.*, 31, 1920, p. 600.
18. Bliss, S. W. *J. Metabolic Research*, 2, 1922, 385-400. Effects of insulin on diabetic dogs.



## EXPERIMENTAL STUDIES IN DIABETES.

### SERIES V. ACIDOSIS.

#### 2. Fat Intoxication.

FREDERICK M. ALLEN, M. D.

*From the Hospital of the Rockefeller Institute for Medical Research,  
New York.*

Preliminary mention of the condition called "fat intoxication" has been made in two former publications.<sup>1,2</sup> The present paper will merely present several records illustrating this condition in dogs.

Dog B2-88 has been previously mentioned.<sup>3</sup> This was a female yellow mongrel, potentially diabetic following partial pancreatectomies in April and May, 1915, but kept in a "border-line" condition for exercise and other experiments. The original weight in good nutritive condition was 14.4 kg. The subsequent diet was mostly mixed, adequate in protein and carbohydrate and not high in fat. It was tolerated generally without glycosuria in consequence of a reduction of weight to 12 or 11 kg.

After April 19, 1916, 100 gm. suet was included regularly in the diet. November 14, this was increased to 150 gm. In addition, 200 gm. of beef lung was fed daily, sometimes with the addition of bread. This diet was eaten and digested well and the animal remained in excellent condition until the termination of the exercise experiments in the early part of January, 1917. During this time there was gain of weight to as much as 16 kg. Glycosuria thus appeared and was checked by reduction of protein and carbohydrate, while the suet was continued. Such diets were well eaten, but by the end of January the weight had again fallen to 12 kg.

About this time the animal began to show weakness and nervous symptoms. There was choreiform twitching of the forequarters and unsteadiness in the hind limbs in standing. These symptoms increased until in February the dog could barely walk. The actual strength was still fair and the spirits excellent. Various articles were added to the diet in the attempt to correct possible deficiencies of either salts or vitamins—raw bones, raw eggs, raw meat, milk and yeast. The low tolerance was responsible for glycosuria whenever a high diet was given, and slight ketonuria was present most of the time with or without glycosuria. The condition was not improved, and extreme unsteadiness and incoordination of the legs out of all proportion to the general strength remained the striking feature throughout this entire period.

By February 14, 1917, the weight had fallen to 8.5 kg. The dog, though emaciated, was still in excellent spirits. It was decided to permit glycosuria in order to observe whether a more liberal diet would alter the parietic condition. Accordingly, the diet was changed to 200 gm. lung, 200

gm. suet, and 75 gm. bread. Heavy glycosuria and moderate ketonuria were continuous after this time. Strength and weight were thus gradually lost, while the nervous condition also gradually became worse. A portion of the final record is given in Table 1.

February 24, the dog was very weak, but the loss of muscular control was out of all proportion to the loss of strength. The animal made fairly vigorous efforts to stand, but was unable to do so because of twitching and incoordination. With the dog resting flat on her side, there were continual alternating twitchings of all the limbs, without pain or any of the disturbances of ordinary convulsions. February 25, the weakness was more advanced, but the twitchings still present. The previous acetoneuria and lipema had cleared up. Death occurred on this day from extreme weakness, the body weight being 8 kg.

The autopsy was negative as usual. The pancreas remnant weighed 2.75 gm. and microscopically showed fibrous and hydropic changes. The liver was moderately large, weighing 401 gm., and was of nutmeg character, both grossly and microscopically. The kidneys together weighed 64 gm. Slight interstitial nephritis was present, together with the Armani vacuolation of Henle tubules. The other viscera were negative. The brain and spinal cord were apparently normal and the cerebrospinal fluid moderate in quantity. Nothing abnormal was found microscopically in routine stains of sections of the cerebrum, cerebellum, medulla, thoracic and lumbar cord and their nerve roots, and of the sciatic nerve.

Dog D4-14, a female fox terrier mongrel, aged three years, weighed 9 kg., in a state of moderate obesity. July 13, 1916, partial pancreatectomy was performed as previously described.<sup>4</sup> Glycosuria began only in October, probably in consequence of fibrosis in the large pancreas remnant. Beginning October 1, the diet included 150 gm. suet, with such quantities of lung as the dog would eat. The weight had fallen to 5.7 kg., and the suet was fed forcibly when necessary in the attempt to build up weight. The weight, nevertheless, fell to a minimum of 5 kg.

Though the strength remained fair, muscular incoordination gradually developed. The animal, though lively to the point of playfulness, was shaky in standing, fell down when attempting to run or turn quickly, and was entirely unable to rise on her hind legs. Later the front legs were equally involved, so that the dog when lying down had difficulty in rising to her feet; she staggered drunkenly in walking, and on attempting to run, her fore legs gave way so that she fell and bumped her nose. Struggling up, with apologetic wagging of her tail, she would repeat the performance, and with further difficulties often cried sharply, not from pain but merely in distress at her own clumsiness.

Typical diabetic cachexia then came on, and ulcers of gangrenous or trophic character developed on the legs. The dog was therefore killed on December 11, the ataxia being present to the end. The autopsy, as usual, gave no explanation of the nervous condition.

The chemical findings throughout were also those of ordinary diabetes. Table 2 contains part of the later records, when ketonuria was heaviest, and shows that acidosis was not an explanation.



TABLE 1  
Dog B2-88

Date	Weight kg.	URINE					BLOOD			Diet	
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Total Acetone mg.	Sugar mg. per 100 cc.	Nitro- prusside Reaction	Lipemia Qual.		
Feb 14	.....	508	22.42	4.64	Faint	0	333	Neg.	Neg.	200 gm. lung, 200 gm. suet,	75 gm. bread.
" 15	8.4	200	2.12	2.39	Slight	90.0	.....	.....	.....	200 " " 100 " "	75 " "
" 16	.....	498	15.16	4.71	"	30.0	.....	.....	.....	200 " " 100 " "	75 " "
" 17	8.2	336	6.85	4.60	"	30.0	.....	.....	.....	200 " " 100 " "	75 " "
" 18	.....	818	38.40	7.36	"	120.0	.....	.....	.....	200 " " 100 " "	75 " "
" 19	8.4	646	36.40	9.64	"	80.0	500	Neg.	Faint	200 " " 200 " "	75 " "
" 20	8.6	938	31.30	8.80	"	160.0	625	"	Slight	200 " " 200 " "	75 " "
" 21	.....	764	22.16	9.17	Mod.	168.1	.....	.....	.....	200 " " 200 " "	75 " "
" 22	.....	282	9.65	.....	"	.....	.....	.....	.....	300 " " 200 " "	" "
" 23	8.0	240	2.60	2.98	Heavy	122.5	.....	.....	.....	300 " " 200 " "	" "
" 24	.....	556	22.00	6.26	Mod.	120.0	.....	.....	.....	300 " " 200 " "	" "
" 25	.....	96	2.30	0.85	Slight	.....	250	Neg.	Faint	Found dead at midnight.	" "



Dog D4-24, a brown shaggy male mongrel, aged 4 years, had lived on carbohydrate diet in another department of the Institute for three years, and was very slightly obese at a weight of 15.9 kg. After a preliminary observation period on bread and soup diet, September 12-17, 1916, a fasting period was imposed, September 18-October 1. No appreciable acidosis developed. Sample days from the record are shown in Table 3.

In an unsuccessful attempt to increase ketosis, 300 gm. suet mixed with talcum powder was fed daily, October 2-4. From October 5 onward, a regular diet of 150 gm. beef-lung and 250 gm. suet was given. The dog immediately began to refuse the suet, which had to be fed forcibly. After October 9, the entire diet had to be fed forcibly, and practically continuous watching was necessary to prevent vomiting. Diarrhea was satisfactorily controlled by bone-meal and talcum powder, and the feces were well formed and not very bulky.

By October 15, the weight had fallen to 12 kg. The loss of strength was much greater, but the intoxication was shown chiefly by the usual muscular symptoms. On this day the dog could scarcely walk on account of staggering. When resting on his side in the cage, he exhibited continual twitchings of all four limbs and also of the head, but these became less when he was taken out of the cage and petted. Consciousness was clear as usual, and the animal took interest in surroundings and events in a manner which contrasted with his helplessness. The respiration was normal and slow (16 per minute at rest), the pulse approximately normal (120 to 170 per minute), the rectal temperature subnormal (37.8 or 37.9° C.). The same diet was continued on this day.

October 16, the temperature, pulse, respiration and muscular symptoms were similar, but the weakness was somewhat greater. Instead of the former diet, carbohydrate in the form of 150 gm. bread and 50 gm. glucose was fed in the attempt to save the animal. The dog did not appear to be in dangerous condition when last seen that evening, but was found dead the next morning. The autopsy was entirely negative grossly and microscopically. On account of postmortem changes, the study of the pancreas was unsatisfactory, and no sections were made of the nervous tissues.

This case is noteworthy for two features. First, it occurred in a normal animal, without diabetes or any pancreatic operation, and reached a fatal stage before there was any serious emaciation. The body at autopsy still contained abundant fat in all regions. Second, the hyperglycemia observed in human subjects on excessive fat rations<sup>5</sup> was also in evidence here. There was a corresponding reduction of carbohydrate tolerance, as shown by the increased hyperglycemia and faint glycosuria following the carbohydrate feeding of October 16. The autopsy blood, though taken hours after death when the animal was cold and in rigor, still showed 0.175 per cent. sugar by Benedict's method.

Dog D4-27, a black male mongrel, aged 2 years, had been born on the Institute farm and lived on cereal mixtures all his life. He was in good nutrition at a weight of 12 kg. After a preliminary period of urinalyses on this diet from September 29 to October 2, 1916, a diet of 800 gm. beef-lung was given from October 2 to 6.



October 7 to 9, only 300 gm. of suet was fed daily. As distaste for the fat developed, the diet was changed to 150 gm. lung and 200 gm. suet, which was mostly fed forcibly, but retained and digested well until December 6. The weight rose as high as 12.5 kg., November 8 to 13, then fell with failing digestion to 11.5 kg.

The dog fasted from December 7 to 10 in order to rest the digestive function, then received nothing but 100 gm. of suet daily from December 11 to 19, and 200 gm. of suet daily from December 20 to 26. The weight thus fell to 9.9 kg. The dog retained fairly good strength and spirits, but most of the formerly luxuriant coat of hair had fallen out by this time.

From December 27 to January 4, the diet was 100 gm. lung and 200 gm. suet. The weight fell to 9.6 kg., and toxic symptoms became marked. No eczema, parasitic infection or itching of the skin was evident, but the dog was nearly naked of hair and the rest fell out in bunches wherever grasped. There was partial paralysis of the hind legs and general muscular tremors, not explainable by the degree of general weakness.

January 5, the diet was changed to 200 gm. lung and 100 gm. suet in the attempt to improve the strength by a more adequate protein supply. The condition on this and the following day was the same. January 7, the dog was found sprawled in his cage, unable to stand. The upset water cup and other signs indicated that there had been violent convulsions in the night. The dog was fully conscious and intelligent, appreciated petting, and made strong movements of the body and all four limbs in attempts to stand, which failed because of incoordination. Carrying the animal from the cage to a table brought on a rather violent general convulsion which passed off after a minute or two without unconsciousness. The rectal temperature was 37.7° C.; the pulse 128 per minute, strong and regular; the respiration slow and quiet, 12 per minute. Shortly before noon a subcutaneous injection was given of 750 cc. of 0.85 per cent. NaCl solution containing 40 gm. of Merck glucose. There was no reviving effect. The dog continued to grow weaker and died about 7 P. M.

The final body weight was 8.8 kg. The appearance was of moderate emaciation, but the quantities of fat in different regions were still much greater than in starvation. The muscles were not perceptibly wasted, and their bulk and healthy firmness were opposed to any idea of protein deficiency. Nothing abnormal was observed in the brain, cord or spinal fluid. The viscera all appeared normal, except for fattiness of the liver. The weight of the liver was 426 gm., of both kidneys 59 gm., and of the pancreas 22.1 gm. The spleen, adrenals, thyroid, parathyroids and hypophysis seemed normal in size and otherwise, as in all the other animals. Microscopic sections of all the organs mentioned, fixed in Zenker fluid and stained with eosin-methylene blue (no fat stains or other special technique) showed nothing requiring comment except the following: The liver was markedly infiltrated with fat but otherwise normal. An apparent slight increase of vacuolation in the adrenal cortex suggested possible increase of lipid storage. The kidneys were normal, except for vacuolation in the cells of Henle's loops, which would ordinarily be assumed to



represent glycogen, but in this case probably represented fat accumulation. The pancreas was strictly normal in islands and acini.

As shown in Table 3, the plasma sugar in this dog was uniformly low. The carbohydrate tolerance, however, was reduced as the hyperglycemia and glycosuria exceeded anything that might be expected in a normal dog from such a glucose injection. This reduction of tolerance may be regarded as merely the transitory phenomenon, which is known to follow fasting or carbohydrate-free diet and apparently is not related to deficiency of the pancreas.

Dog D4-30, a female mongrel, aged 3 years, had been born on the Institute farm and lived on carbohydrate diet all her life. The weight was 9.7 kg. in a state of medium nutrition. The urine was collected through a preliminary period on bread and soup diet, from September 28 to October 6, 1916. The diet was then changed abruptly to 50 gm. beef-lung and 200 gm. suet. Though it was soon necessary to feed the fat forcibly, the diet was very well borne, so that by the end of November the weight had risen to 10.2 kg. Slight ketonuria was present, as shown by the partial record in Table 4.

During December, indigestion and vomiting became very troublesome, so that the weight fell to 9.1 kg. On December 18, the dog at this weight appeared fairly well nourished, but was found sprawled helpless in the cage. She was nervous and frightened, but fully intelligent and responsive to petting. There was distinct exophthalmos and dilatation of pupils. The actual muscular strength seemed practically normal, so that when the dog started floundering in attempts to rise she was difficult to restrain. But when the dog was lifted, all four legs were stiffly extended almost straight posteriorly. This spasticity affected the trunk and neck muscles also, but there was no opisthotonus. With the dog at rest on her side, there was continuous tremor of the head and neck muscles, but not of the limbs as in other dogs. The violent attempts to rise terminated in moderate general convulsions. The rectal temperature was 39.9° C., and the pulse and respiration normal except as accelerated by the frequent muscular exertions.

About noon, the dog was fed forcibly 200 gm. of lung, and was given by stomach tube 400 cc. of water containing 50 gm. of glucose. Vomiting was forcibly prevented until 7 P. M. There was considerable absorption, as indicated by the passage of a large quantity of sugar-rich urine, most of which was lost. After 7 P. M., the dog was allowed to drink water, which she soon vomited. The vomitus contained no meat or other solids, but gave a very heavy sugar reaction.

On December 19, the nervous symptoms were still more marked and the dog was dangerously weak. Death occurred just before noon, and autopsy was performed immediately. The gross appearance of the body was normal, except for slight loss of hair. There was abundance of adipose tissue everywhere, and the muscles were beautifully developed. Both lungs showed the typical inflammation which follows aspiration of vomitus, and this accident on December 18 doubtless hastened death appreciably. All the viscera and the nervous system appeared normal.

TABLE 4  
Dog D4-30

Date	Weight kg.	URINE					Diet
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	Total Acetone mg.	
Oct. 1	.....	366	Neg.	11.24	Neg.	.....	Bread and soup.
" 2	.....	320	"	7.62	"	.....	"
" 3	.....	No urine	.....	.....	.....	.....	"
" 4	9.7	452	Neg.	8.50	Neg.	.....	"
" 5	.....	308	"	5.27	"	.....	"
" 6	.....	350	"	6.09	"	.....	"
" 7	.....	370	"	6.88	"	.....	"
" 8	.....	112	"	4.21	"	.....	"
" 9	.....	174	"	6.07	"	.....	"
Nov. 21	10.1	220	"	4.53	Faint	24.2	50 gm. lung, 150 gm. suet.
" 25	.....	170	"	3.20	Slight	.....	50 " 200 "
" 26	.....	232	"	5.07	"	53.4	50 " 200 "
" 27	.....	275	"	3.85	Faint	.....	50 " 200 "
" 28	.....	115	"	.....	Slight	26.5	50 " 200 "
" 29	.....	315	"	5.93	Faint	47.3	50 " 200 "
" 30	.....	251	"	4.47	Slight	.....	50 " 200 "
Dec. 1	10.2	No urine	.....	.....	.....	.....	50 " 200 "
" 2	.....	140	Neg.	1.30	Slight	36.4	50 " 200 "
" 3	.....	38	"	0.72	Mod.	.....	50 " 200 "
" 4	10.4	388	"	1.55	Faint	31.0	50 " 200 "
" 5	.....	140	"	1.64	Slight	46.2	50 " 200 "
" 6	.....	120	"	1.62	"	36.0	50 " 200 "
" 7	.....	No urine	.....	.....	.....	.....	50 " 200 "
" 8	.....	210	Neg.	3.84	Slight	63.0	50 " 200 "
" 9	.....	71	"	0.56	Slight	.....	50 " 200 "
" 10	.....	125	"	3.08	"	25.0	50 " 200 "
" 11	.....	144	"	2.66	Mod.	96.5	50 " 200 "
" 12	10.1	70	"	0.36	Slight	.....	50 " 200 "
" 13	.....	280	"	.....	Faint	19.6	50 " 200 "
" 14	.....	242	"	1.43	"	26.6	Not fed.
" 15	.....	140	"	1.04	"	44.8	"
" 16	.....	292	"	1.34	Neg.	43.8	50 gm. lung, 200 gm. suet.
" 17	.....	108	"	1.37	"	30.2	Plasma sugar 195 mg. per 100
" 18	9.1	45	"	1.16	Mod.	.....	cc. 50 gm. glucose, 200 gm. lung.

The liver weighed 265 gm., the pancreas 15.9 gm., and the two kidneys 50 gm. The usual routine stains showed normal microscopic structure of the adrenals, spleen, parathyroids, hypophysis, cerebrum and cerebellum, medulla, cervical and lumbar cord and nerve roots, sciatic nerve and thigh muscle. The liver showed remarkably little fat vacuolation, perhaps because of the glucose feeding. The kidneys showed vacuolation in cells of Henle's loops, whether from fat or glycogen was undetermined. The thyroid was small in gross, and microscopically was composed of moderate-sized colloid vesicles with a rather unusual richness of cellular tissue between. The pancreas was fully normal in acini and islands.

This dog bears a close resemblance to D4-24 in respect to the early occurrence of fatal intoxication in a non-diabetic animal, and the decided hyperglycemia on the high fat diet. The contrast of both these dogs with the low blood sugar of dog D4-27 may be explainable by their much better nutrition. The glucose tolerance was markedly reduced as usual, without any indication of pancreatic impairment.

Dog D4-47, a brindle female bull terrier, aged about 4 years, was received November 20, 1916, after having lived apparently in the streets. The weight in good nutritive condition was 11.8 kg. A diet of only 50 gm. lung and 250 gm. suet was begun, and diarrhea was controlled by use of considerable talcum powder. By December 6, the weight had fallen to 10.7 kg., and indigestion prevented further continuance of the diet. The dog appeared somewhat unwell and depressed, but became lively with a single day of fasting. After three days of fasting, the dog on December 10 ate 100 gm. of suet voluntarily. This was fed daily as the only diet to December 19, and then increased to 200 gm. daily, which was fed daily (mostly forcibly) till January 23, without serious digestive disturbance. By this time the weight was reduced to 9 kg. and the dog was noticeably, though not dangerously, weak. There was a moderate degree of itching eczema with loss of hair. Ketonuria was only slight and intermittent, as shown in Table 5.

Beginning January 24, a diet of 50 gm. lung and 200 gm. suet was given. By February 12, the weight had fallen to 8.5 kg. The dog was thin and weak, but also showed special weakness and ataxia in the hind legs, different from anything to be found in an animal merely starved to this extent.

Beginning February 13, the diet was increased to 200 gm. suet, 100 gm. lung and 100 gm. bread. The dog at this time was nearly hairless and had itching eczema in an advanced form. There was gain of appetite and weight on the new diet, and the skin eruption was cleared up by sulphur ointment. An incidental experiment was performed by the subcutaneous injection of 0.5 gm. phlorizin on March 10, so as to rob the animal of some of the carbohydrate contained in the diet, but neither acidosis nor fat intoxication resulted. April 11, the diet was increased to 300 gm. lung, 200 gm. suet and 75 gm. bread. By May 14, the weight had risen to 12.2 kg.; the dog was in splendid condition without any remaining signs of intoxication, and was subsequently used for partial pancreatectomy and other experiments.

TABLE 5  
Dog D4-47

Date	URINE				Diet
	Vol. cc.	Sugar gm.	Total N gm.	Total Acetone mg.	
1916					
Nov. 24.....	140	Neg.	2.14	Neg.	50 gm. lung, 250 gm. suet.
" 25.....	340	"	6.22	"	50 " " 250 " "
" 26.....	92	"	2.43	"	50 " " 250 " "
" 27.....	422	"	6.63	"	50 " " 250 " "
" 28.....	562	"	4.38	"	50 " " 250 " "
" 29.....	612	"	4.10	"	50 " " 250 " "
" 30.....	567	"	5.73	"	50 " " 250 " "
Dec. 1.....	715	"	7.01	"	50 " " 250 " "
" 29.....	61	"	1.52	"	200 gm. suet.
" 30.....	168	"	3.14	"	200 " "
" 31.....	86	"	1.15	"	200 " "
1917					
Jan. 1.....	192	"	1.56	"	200 " "
" 2.....	Urine lost				200 " "
" 6.....	496	Neg.	5.65	49	200 " "
" 7.....	371	"	2.89	57	200 " "
" 8.....	572	"	1.77	17	200 " "
" 9.....	590	"	1.71	53	200 " "
" 10.....	412	"	1.52	33	200 " "
" 11.....	516	"	1.55	41	200 " "
" 12.....	640	"	2.75	Neg.	200 " "
" 13.....	262	"	1.65	"	200 " "
" 14.....	325	"	2.43	"	200 " "
" 15.....	805	"	2.74	"	200 " "
" 16.....	628	"	1.76	Faint	200 " "
" 17.....	815	"	3.59	Slight	200 " "
" 18.....	745	"	2.83	"	200 " "
" 19.....	661	"	1.92	112	200 " "
" 20.....	370	"	1.04	52	200 " "
" 21.....	711	"	1.73	142	200 " "
" 22.....	546	"	1.69	22	200 " "
" 23.....	200	"	2.83	152	200 " "
" 24.....	160	"	1.27	Mod.	200 " " 50 gm. lung.
" 25.....	172	"	3.72	49	200 " " " " "
" 26.....	414	"	4.51	393	200 " " " " "
" 27.....	441	"	2.92	25	200 " " " " "
" 28.....	380	"	2.22	Neg.	200 " " " " "
" 29.....	156	"	1.21	"	200 " " " " "
" 30.....	254	"	3.30	98	Not fed.
" 31.....	505	"	2.35	303	" "

This dog was cachectic at the time of incipient fat intoxication in February, and accordingly had low blood sugar. The principal teaching of the experiment is that complete recovery from the early stages of fat intoxication is possible on mixed diet. This diet may apparently contain considerable fat, provided the protein and carbohydrate are ample.

Dog D4-48, a male bull terrier, aged about 3 years, was received on November 20, 1916. The weight in good nutritive condition was 14.6 kg. A diet of 50 gm. lung and 250 gm. suet was continued to December 8, when fasting was begun.

Beginning December 11, only 100 gm. suet was fed daily to December 20. The diet was then changed to 50 gm. lung, 50 gm. bread and 100 gm. suet. On subsequent days the suet was increased to 150 and then to 200 gm. Edema of the legs began to be noticeable. Itching eczema also appeared and resisted treatments with sulphur ointment. Curiously, the appetite for suet was not lost and no forcible feeding was necessary. Digestion was satisfactory and weight was lost only to 12 kg. The general strength remained excellent, but the hind legs became spastic and slightly incoordinate. The addition of one raw egg to the daily diet made no perceptible difference. By February 1, there was indigestion and perceptible weakness, though the weight was no lower.

A liberal diet was then begun, consisting of 200 gm. lung, 250 gm. suet and 200 gm. bread. Weight was thus gained steadily, and the skin eruption cleared up. By April, the dog was obese at a weight of 16.75 kg., and was used for pancreatectomy. A stilted action of the hind legs remained as a permanent effect, nevertheless.

Several control experiments were performed. One of these was in dog D4-28, previously mentioned.<sup>6</sup> This was one of the animals which was known to have lived on carbohydrate during the entire previous lifetime, and was subjected to fasting and fat diet immediately following partial pancreatectomy, in the attempt to produce acidosis. The proposed publication of the protocol is omitted, because it merely duplicates some of the records already given. The first feature is that the sudden deprivation of carbohydrate, together with pancreatectomy to the extent of producing potentially severe diabetes, did not create any special tendency to acidosis. The dog was then kept on low mixed diets to demonstrate the control of diabetes by undernutrition. The protein was as low as in the experiments which led to fat intoxication, and it was proved that a dog could maintain vigorous health with urinary nitrogen excretion between 1 and 2 gm. daily. Small quantities of carbohydrate and fat were included in the diet. This record<sup>6</sup> is one of those which show that neither general undernutrition nor protein deficiency is responsible for the toxic symptoms found in other dogs.



TABLE 6  
Dog 14-48

Date	Weight kg.	URINE				Diet
		Vol. cc.	Sugar gm.	Total N gm.	Nitro- prusside Reaction	
Nov. 21, 1916	.....	758	Neg.	.....	Neg.	Bread and soup.
" 22	.....	515	"	.....	.....	"
" 23	14.6	No urine		.....	.....	50 gm. lung, 250 gm. suet.
" 24	.....	510	Neg.	10.46	Neg.	50 " " 250 " "
" 25	.....	No urine		.....	.....	50 " " 250 " "
" 26	.....	184	Neg.	5.30	Neg.	50 " " 250 " "
" 27	.....	295	"	5.98	"	50 " " 250 " "
" 28	.....	162	"	6.08	Faint	50 " " 250 " "
" 29	.....	174	"	5.94	Slight	50 " " 250 " "
" 30	.....	256	"	11.26	Doubtful	50 " " 250 " "
Dec. 1	14.1	No urine		.....	.....	50 " " 250 " "
" 2	.....	140	Neg.	6.30	Neg.	50 " " 250 " "
" 3	.....	No urine		.....	.....	50 " " 250 " "
" 4	.....	258	Neg.	7.97	Neg.	50 " " 250 " "
Jan. 27, 1917	12.7	168	Neg.	3.98	Neg.	50 gm. lung, 200 gm. suet, 200 gm. bread.
" 28	.....	255	"	3.57	"	50 " " 200 " " 200 " "
" 29	12.9	260	"	3.04	"	50 " " 200 " " 200 " "
" 30	12.8	508	"	7.16	"	50 " " 200 " " 200 " "
" 31	12.6	220	"	2.36	"	50 " " 200 " " 200 " "
Feb. 1	12.2	200	"	2.41	"	200 " " 200 " " 200 " "
" 2	12.6	338	"	7.65	"	200 " " 200 " " 200 " "
" 3	12.5	264	"	1.91	"	200 " " 200 " " 200 " "
" 4	.....	172	"	3.10	"	200 " " 200 " " 200 " "
" 5	12.2	310	"	4.96	"	200 " " 200 " " 200 " "
" 6	12.4	545	"	11.88	"	200 " " 200 " " 200 " "

Several other conceivable explanations can also be excluded. The condition was not a spontaneous disease, for it was limited strictly to the fat-fed animals at different times. The terminal state was somewhat suggestive of "dumb" rabies, but the duration was often too long and recovery from the milder stages was possible. It was also independent of distemper, and was altogether different from the chorea sometimes found in dogs. It was not governed by the previous diet, for though it was observed oftenest in dogs which had formerly lived chiefly on carbohydrate, other dogs seemed to be equally susceptible. It was not a mere symptom of weakness, for though it occurred sometimes in cachectic animals, some of the most striking instances were in dogs which still retained nearly normal body weight and muscular power. It bore no relation to diabetes or pancreatectomy, for it occurred alike in normal dogs and those with either potential or active diabetes. It could not be ascribed to the trivial acidosis, for no such symptoms were exhibited by dogs which developed severe acidosis. According to Tallqvist's hypothesis, anemia might result from such excessive fat diets and fatty indigestion. No blood counts were made, but the corpuscle volume in centrifugalized blood samples indicated no anemia as the cause of the disturbance. The trouble was also not a deficiency of vitamins, salts or other accessory food elements, for the addition of these brought no improvement.

The name "fat intoxication" seems to be warranted by the evidence that the cause is an excessive proportion of fat in the diet. As different dogs seem to differ somewhat in susceptibility, and the time limits of the experiments are also variable, the exact quantities or proportions of fat necessary to produce the condition could not be determined. The chief or only cause of failure in attempts to produce the intoxication seems to be failure of digestion. The long periods of fat stuffing and prevention of vomiting make the experiments very tedious to carry out successfully. The quantities of other foods must be kept sufficiently low, for both indigestion and intoxication can often be cleared up by additions of sufficient protein or carbohydrate without reduction of the fat intake. There is no evidence whether the intoxication is due to the simple overload of fat or to abnormal products of disturbed digestion.

*Remarks.*

The nature of the nervous symptoms in fat intoxication is unknown, and they probably have no direct relation to any human disease. The skin condition of the dogs was also not closely investigated, and was possibly not uniform; i. e., it was perhaps parasitic in some instances and non-parasitic in others. The skin changes in all the animals, however, even when not mentioned specifically in the protocols, were sufficient to justify the statement that an injury was evident, in the form either of a spontaneous eruption or a lowered resistance to infection, and to a degree far surpassing what is found with either diabetes or simple malnutrition. As the relation of diet to human eczema is well recognized, at least in infants, this association in dogs is not surprising.

The chief instructiveness of the experiments is in connection with artificial schemes of diet. There is a widespread tendency to conceive diabetic diets solely in terms of nitrogen balance and ketosis. The question of proper balancing of the diet was necessarily encountered very early in our investigation of diabetes. Dogs furnish an interesting experimental opportunity, because they are carnivorous animals practically immune to any dangerous ketosis. Therefore it should be possible to push their so-called ketogenic-antiketogenic ratios to a corresponding extreme, reducing the glucose factor much lower than in man. Tests readily prove that this supposition is contrary to fact.

The simplest observation can be made upon fasting animals. As the fasting organism must live strictly upon its own reserves, and an obese animal can survive longer fasting than a thin one because of the extra fat supply, it might be supposed that an outside supply of fat would be welcome and that this extra source of energy would be the means for a much longer survival. The truth is the reverse. Animals of every species, no matter how fond of fat in their natural diet, soon develop an unconquerable loathing for fat when this is fed in a pure or relatively pure form, and will endure any degree of starvation in preference. If the fat is given forcibly, extreme measures soon become necessary to prevent vomiting, and if such measures succeed, the remarkable cessation of digestive function nevertheless results in the passage of practically the unchanged fat in the feces. This

phenomenon may be merely a peculiarity of digestion, or some teleologic mechanism for protecting the body against harm.

A further experimental step is to supply approximately the minimum requirement of protein and make up a high caloric diet by addition of fat alone. A serious acidosis, which was the first purpose of these attempts, ordinarily does not develop in dogs, but the result is first indigestion and then intoxication, as described in this paper. These troubles may be prevented by two methods, either a reduction of fat to make an undernutrition ration, or an increase of protein or carbohydrate so as to balance the high fat.

Likewise, human patients, free from glycosuria, may receive very one-sided fat diets which cause only slight ketonuria, if any. They do not feel as strong or well as on more liberal allowances of protein and carbohydrate. Theorists may attribute the malaise to a masked ketosis, but the experiments with dogs are opposed to this interpretation. The anorexia, indigestion and lack of strength on diets extremely low in protein and carbohydrate and high in fat may indicate a form of injury independent of acidosis. Such diets are, at least, more uncomfortable than undernutrition in both patients and animals, and it is doubtful if any human beings will remain very long on some of the most radical diets that have been proposed. The dog experiments show the fatal consequences that may follow if the earlier warning symptoms are ignored and the fat diets carried to extremes.

#### *Summary and Conclusions.*

Dogs fed on diets high in fat, not balanced with sufficient quantities of other foods, develop first indigestion and later intoxication. Besides a skin eruption with loss of hair, the chief symptoms are various grades of ataxia and muscular incoordination, and twitchings which before death may reach the point of convulsions. This intoxication is independent of both acidosis and diabetes, though some animals show the hyperglycemia and lowered glucose tolerance which seem to result from excessive fat diets. No organic lesions are found in the nervous system or viscera. The intoxication seems to be due entirely to the absolute and relative excess of fat. These results in a species comparatively immune to ketosis indicate that ketosis is not the only harmful feature to consider in connection with high fat diets.

## REFERENCES.

1. *Amer. J. Physiol.*, 42, 1916-17, 583-584. Observations concerning fat feeding.
2. *Amer. J. Med. Sci.*, 153, 1917, 347. The role of fat in diabetes.
3. *Amer. J. Med. Sci.*, 161, 1921, 187. The effects of exercise.
4. *J. Metabolic Research*, 1, 1922, 172. Pancreatitis in the etiology of experimental diabetes.
5. Weeks, Renner, Allen, Wishart. *J. Metabolic Research*, 3, 1923, 317-364. Observations on fasting and diets in the treatment of epilepsy.
6. *J. Exper. Med.*, 31, 1920, 581. Control of experimental diabetes by fasting and total dietary restriction.



---

---

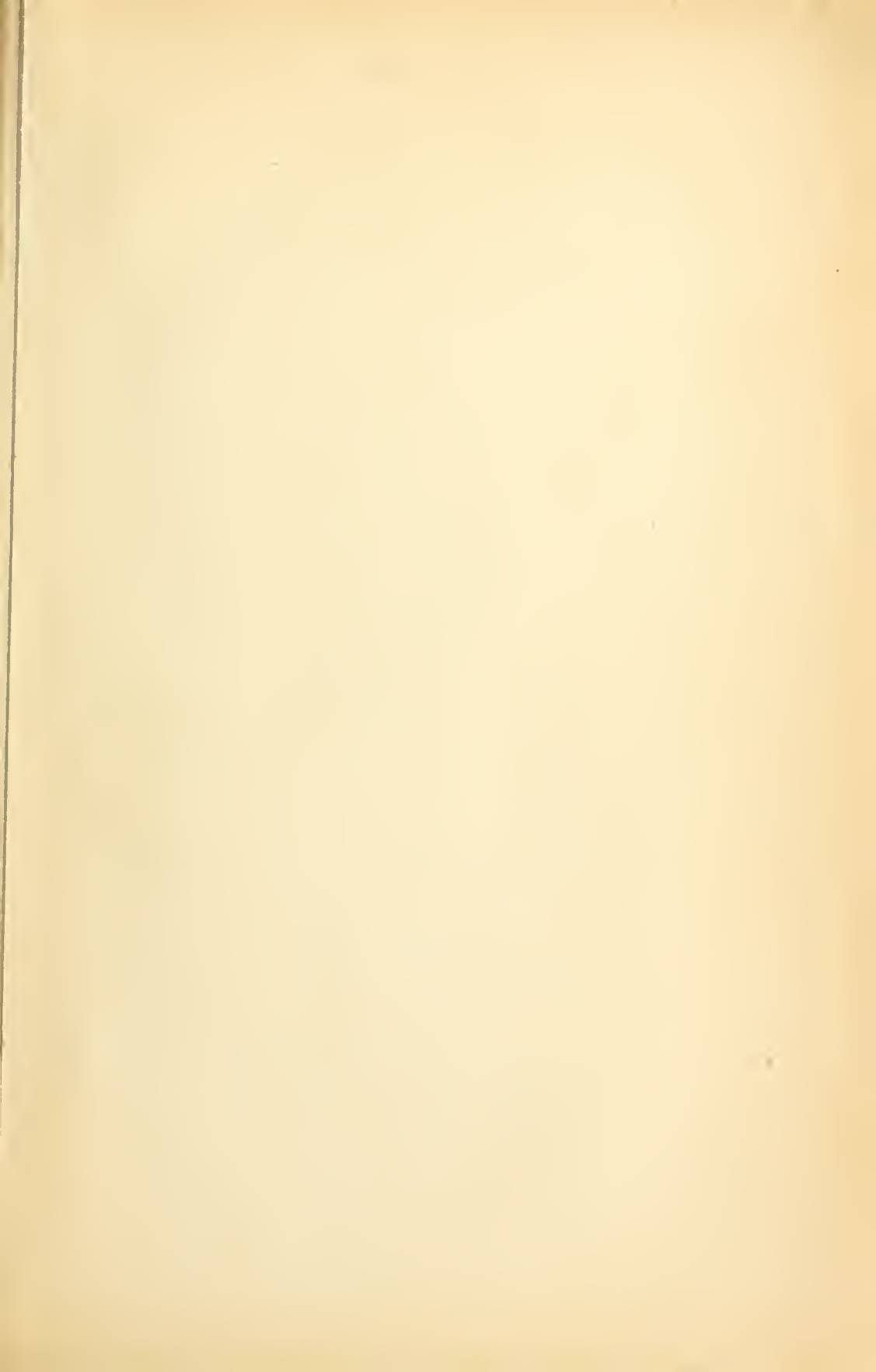
## *Advertisement*

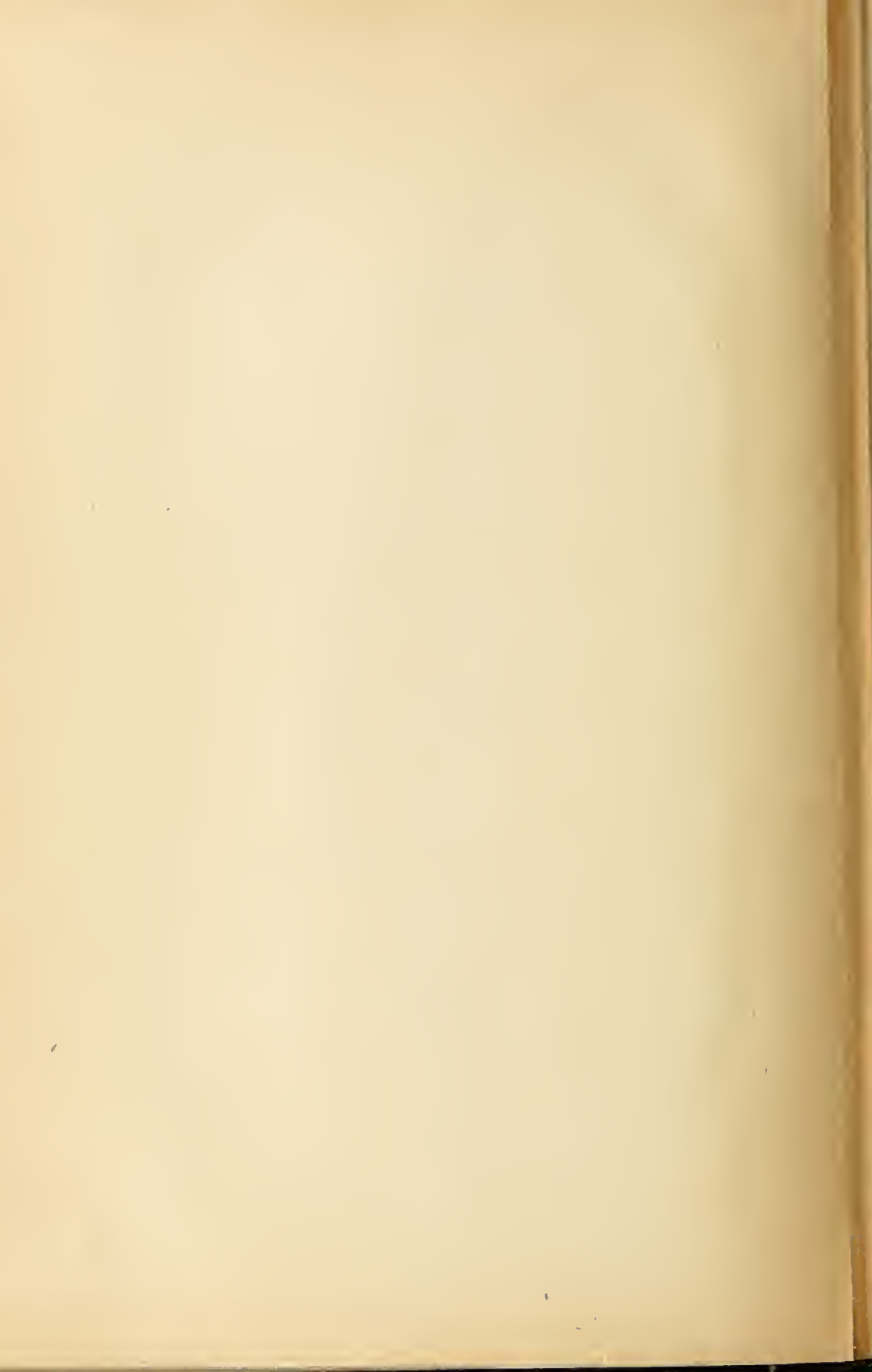
---

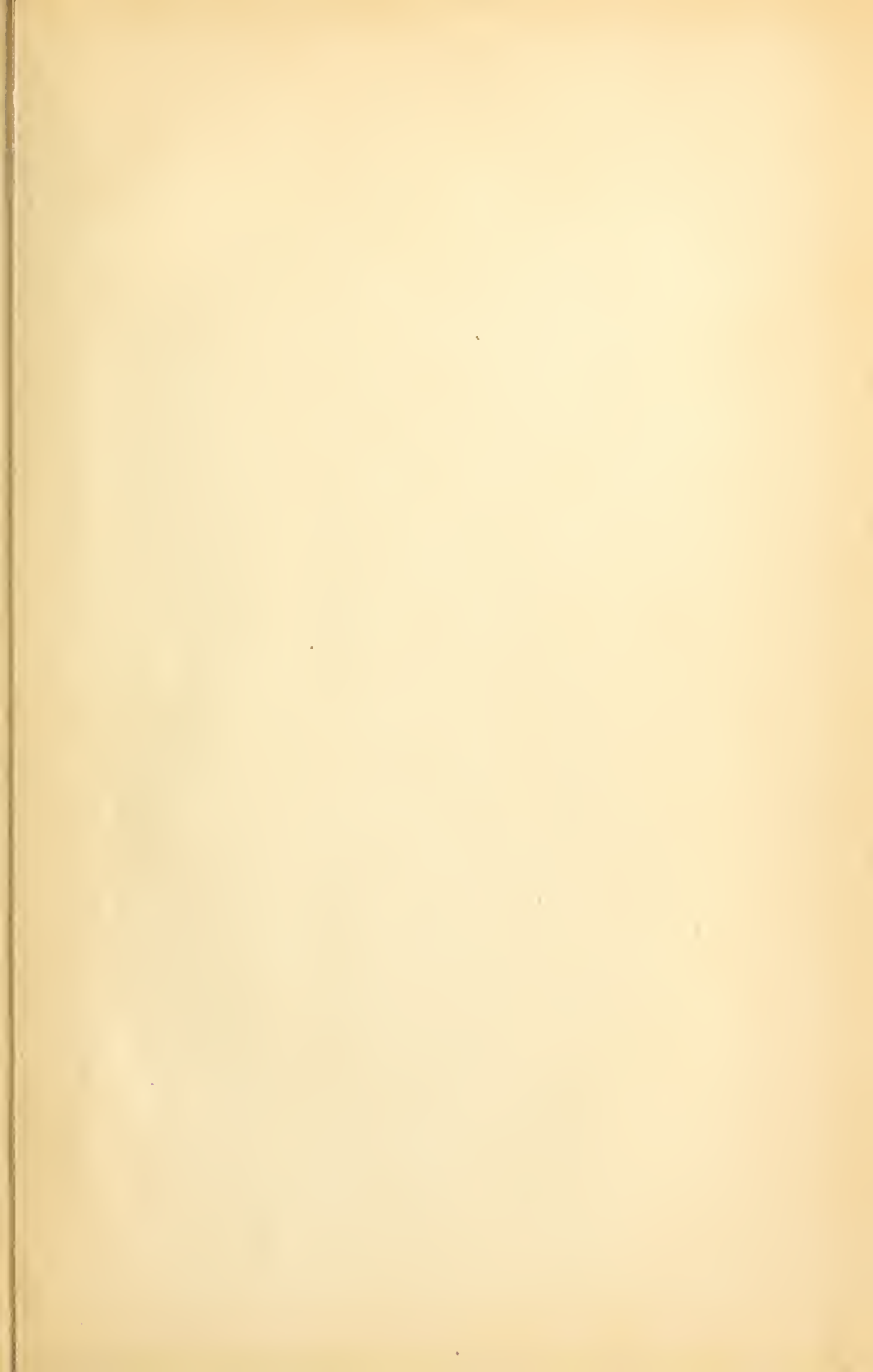
THERE are several unfilled orders for Nos. 1, 2 and 3 of volume 1 of this Journal (Jan., Feb. and March, 1922). Persons having extra copies of these numbers which they are willing to donate or sell will confer a favor by communicating with the editor.

---

---











~~Author~~ <sup>1923</sup> Journal of Metabolic Research  
~~Title~~ Vol 3 - 1923.

Biological  
& Medical

University of Toronto  
Library

Biological —  
& Medical  
Serials

DO NOT  
REMOVE  
THE  
CARD  
FROM  
THIS  
POCKET

Acme Library Card Pocket  
Under Pat. "Ref. Index File"  
Made by LIBRARY BUREAU

